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Reserve. A Summary of Current Program and
Preliminary Report of Progress //

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CROSS-SPECIES

ANIMAL RESEARCH 1/

of the

United States Department of Agriculture
and Cooperating Agencies, 73a

Containing:

Animal Biology	1
Miscellaneous Infectious and Non-Infectious Diseases of Animals.	30
Treatment for Removal or Control of Parasites of Domestic Animals.	60
Miscellaneous Parasites and Parasitic Diseases . . .	75
Nutrition of Animals as Affected by Properties and Characteristics of Soils and Plants.	86

1/ This research work is related to that reported in the separate
commodity reports for Dairy; Poultry; Sheep and Wool; and
Livestock.

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UNITED STATES DEPARTMENT OF AGRICULTURE
Washington, D. C.
January 30, 1963

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SEP 9 - 1964

C & R-PREP

ANIMAL BIOLOGY
Animal Husbandry Research Div., ARS

Problem. The extent of applicable results in animal husbandry research is severely limited by a paucity of basic information on the genetics, nutrition and physiology of our livestock. The basic research in progress has resulted primarily in revealing the inadequacy of our information regarding the functional processes within the animals we are trying to control and develop for human use. Basic research is required in such fields as animal cell metabolism and reproduction, enzymology, physiological bases for heredity and microbiology of the rumen and intestines. Results of such studies provide the basis for additional research applied to the husbandry of each type of livestock.

USDA PROGRAM

This area consists of basic research conducted by geneticists, biochemists, physiologists, and nutritionists. It includes studies in the Pioneering Laboratories on somatic variations of red cell antigens, on the nature of the specificity of antigens and antibodies, and on methods and theories of population genetics. Research in reproductive physiology is in progress to determine the biochemical composition and the defensive mechanisms of the uterus. Still other physiological investigations are involved with the response of mammary tissue to invasion by infectious agents. One study of long standing has the primary objective of describing, fully and in detail, the gross and microscopic anatomy of the domesticated fowl. Research on the metabolic role of vitamin B₁₂, the investigation of unidentified nutrients in food and feed, and biological and chemical studies of rumen metabolism are also being undertaken. The work is conducted at Beltsville, Maryland, East Lansing, Michigan, and in cooperation with the Indiana, Maryland, Michigan, and Wisconsin Agricultural Experiment Stations.

The Federal scientific effort devoted to research in this area totals 11.2 professional man-years. Of these, 5.5 professional man-years are in genetics, 2.0 in nutrition, 3.5 in rumen function, and .2 in program leadership.

There are two grants involving Public Law 480 funds in foreign countries financing research related to animal biology. One is with the Polish Academy of Science, Jabtonna, Poland. The project is on the secretion of anterior pituitary hormones and ovulation in small ruminants and is supported for three years (1960-1962) by \$11,495 equivalent in Polish zlotys. The second project is with the Institute Espanol de Fisiologia y Bioquimica, Madrid, Spain, and is entitled "Study of Metabolism of Zinc in Living Organisms by Means of Zinc 65." It has a duration of four years (1961-1964) and is supported by \$35,277 equivalent in Spanish pesetas.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State experiment stations reported a total of 92.8 professional man-years of which 13.7 are in genetics, 19.1 in physiology, 41.3 in nutrition, and 18.6 in rumen function.

Drosophila, *Tribolium*, mice, guinea pigs, and other laboratory animals are being used to test genetic principles and new concepts in breeding systems. Blood group research includes work on the chemical nature of the antigen-antibody relationship as well as inheritance of serum proteins. Research designed to gain an understanding of fundamental physiological principles includes investigation of various phases of the problem of hormonal control of the reproductive process in the female. Other research is concerned with the overall problem of improving semen production, evaluation, and preservation. Additional physiological research is conducted on biochemical and fundamental physiological changes occurring with genetically variable growth and the relative significance of the various parts of the alimentary tract with respect to transfer of certain minerals.

The animal requirements for specific nutrients and various factors which influence nutrient requirements and utilization pathways are under investigation as follows: (1) Essential macro and micro mineral nutrients and the interrelations and interdependences among them. (2) The influence of factors such as vitamins, hormones, and antibiotics on animal metabolism as reflected by general growth, blood, and liver composition. (3) Nutritional factors involved in certain toxicity problems such as high molybdenum or high selenium levels in certain feeds together with the basic metabolic pathways concerned. (4) The metabolism in animal systems of various fission products.

The fundamental physical and chemical factors responsible for the development of the functioning rumen are under study as are the basic factors influencing the fermentation of feedstuffs in the rumen. The plant constituents which contribute to bloating and the physiological factors in the rumen which are involved in bloat are the primary factors being studied.

There is very little research by industry in the field of animal biology where the major objective is agricultural. A small amount of activity amounting to not more than one or two professional man-years has been undertaken on the effect of antibiotics as related to bloat and rumen digestion.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Genetics

1. Methods and Theories of Population Genetics.

Experimental evaluations of the methods and theories of population genetics are underway at Lafayette, Indiana, in cooperation with the Population Genetics Institute of Purdue University.

a. Tribolium Studies.

Detailed analyses of the effects of mating systems and selection methods on a heterotic trait of Tribolium castaneum, a flour beetle, have been completed. All combinations of two selection methods (mass and random) and the five mating systems (phenotypic assortative, phenotypic disassortative, inbreeding, outbreeding, and random) were investigated through eight generations in two replicates from the same foundation population. One-hundred twenty offspring from each of the twenty populations were individually measured for 14-day larval weight in each generation. A terminal test was made by repeating the two replications of the random mated - random selected subclass and by crossing the two replications from several of the subclasses of generation eight. Also, three mass-selected subclasses were continued in one replication through the twelfth generation. Results are based on mass (or individual) selection and random (or no) selection in closed populations reproduced from a maximum of twenty pairs of parents.

As reported previously, 14-day larval weight showed a high degree of hybrid vigor or heterosis (33.6%) in crosses of stock used to form the population. Heritability of 14-day larval weight in the initial untreated generation was estimated to be $.150 \pm .031$ from the regression of offspring on sire.

The average realized heritability in the mass-selected subclasses over the eight treated generations was .155. A large and highly significant maternal effect was proved to be present. This maternal effect caused a significant upward bias in heritabilities estimated from regression of offspring on dam and regression of offspring on midparent. Heritability did not change significantly over generations, and sex-linkage was not detected. There was no significant difference in heritability estimates between selection methods. Differences in heritability estimates among mating systems were significant only at the 10% level or probability, and apparently resulted from a differential effect of the maternal factor on estimates for assortative and disassortative mating. Realized heritabilities for both replications of the inbred mating system were significantly lower and realized heritability for one replication of the outbred mating system was significantly higher than average for the mass-selected subclass.

Analysis of mean larval weights of sexes in treatment groups over generations showed highly significant effects of selection method (S), mating system (M) and sex (D). Also highly significant were the interactions of generation (G) XS, GXM, SXM, and GXMxD. Analysis within each generation showed that only the sex difference was significant in the untreated generation, but the effects of S, M, and D were highly significant in every treated generation except that D was not significant in one generation. None of the interactions were significant in any generation except that SXM was significant in the last generation at the 5% level of probability.

Analysis over generations of the log transformations of subclass variances indicated no significant effect of sex, selection method or generation but a highly significant effect of mating system. Multiple comparisons of mating systems revealed that the inbred mating system had significantly larger variance than the other four mating systems. No other differences were significant. Because of the trait's low heritability, the expected increase of variance in assortative mating would be quite small and unlikely to be detected as a difference over all generations. However, this mating system did have the largest variance in generation eight.

The Newman-Keuls multiple comparison test was applied to the mean differences between subclasses of generation eight and the terminal test. Based on the average of the two replications of generation eight, the mass-selected outbred subclass showed a highly significant superiority for large larval weight in every comparison except those with the cross between its own two replications (not significant) and with the mass-selected, assortatively mated subclass (significant). These three groups were followed in order of merit by four mass-selected groups (random-mating, the cross of the two replications of inbred mating, disassortative mating and inbred mating) and all random-selected groups. Inbreeding showed a highly significant inferiority to all other mating systems under mass-selection. The cross between its replications ranked above (not significantly) only one other mating system without crossing. The cross of inbreds showed a highly significant amount of heterosis and a larger amount of superiority over the cross between the random-selected inbred replications. The latter cross also showed highly significant heterosis but this only recovered the loss due to inbreeding. This is shown by the exact equality of the crosses between replications of the random-selected inbred subclass and the random-selected, random-mated subclass. Inbreeding was no aid to selection as is shown by the fact that adjustment of the mass selected inbred subclass for loss due to inbreeding is insufficient to raise it to the level of the random-mated subclass.

It is important to note that the superiority of outbreeding over the other noninbred mating systems was not due to the small differences in inbreeding coefficients. For changing this trait, it does not appear

that phenotypic assortative, random and phenotypic disassortative mating were greatly different. They ranked in this order in generation eight but the former happened to be higher and the latter lower in the initial generation. Replications of these subclasses were not crossed in the terminal test. One replication of these subclasses, not different in generation eight, were continued through generation twelve. In generations eleven and twelve, the means for assortative mating were much larger than those for disassortative and random mating, and the differences were highly significant. The latter two systems appeared to be approaching a plateau after generation nine, but the number of generations is insufficient to warrant a conclusion.

b. Mice Studies.

Three cases of unusual reproduction have been observed in mice. Superfetation (conception during pregnancy) has been reported as a rare event in mice and most other mammals. Two of the cases involve the production of two litters after the female was separated from the male. Superfetation and delayed implantation is strongly indicated in one case and delayed implantation in the other. The third case probably resulted from superfetation, with or without a delay in implantation.

In the long-term experiment on selection for white from a colored strain of mice, eight dark-eyed all white males (no females) have now been obtained from seven different sires and eight different dams. One all white male produced an all white son. Thirty-nine full brothers and 26 full sisters of all white males show various amounts of color. Definite progress is being made toward the goal of an all white population which would be the ultimate limit of selection. This experiment was initiated at Mt. Hope farm by Dr. H. D. Goodale in 1931 and is being continued here. Individual mice which have descended from the colored foundation population without selection have from none to a few white hairs on the forehead with the balance of the integument being colored.

2. Basic Research on Blood Antigens and Antibodies.

a. The Nature of Antigenic Specificity.

Studies on the red cell agglutinin of chickens designated "Hi" were continued. Additional evidence was obtained that the "Hi" agglutinin is affected both by the action of estrogen and the genotype of the bird. The restriction of agglutinability to the red cells of sexually mature females suggested that injection of estrogen into males regardless of their age would result in the red blood cells of such birds becoming agglutinable. Furthermore the use of immature birds of either sex or adult males permitted additional data to be

obtained to determine whether the "Hi" agglutinin is affected by heredity and if so to help determine the mode of inheritance.

Injections of diethylstilbestrol begun in genetically competent young chickens at one month of age or older or genetically competent males, regardless of age, resulted in the transformation of the red cells of these birds to a state in which they may be specifically agglutinated with extracts of Lathyrus cicera or Pisum arvense. Progeny test data best fit the hypothesis that the "Hi" agglutinin is under the control of a single autosomal gene.

b. In Vitro Uptake of Fe⁵⁹ in Adult and Immature Human Red Cells.

Studies were initiated to determine whether Fe⁵⁹ could be incorporated into erythrocytes in vitro. Such a method was needed to follow the development and differentiation of avian red blood cells and in particular the "Hi" substance. In vitro incorporation of Fe⁵⁹ in chicken red cells as well as in turkey, pigeon, and human red cells was achieved using Ferric⁵⁹ citrate in the presence of oxygen. The incorporation appears to favor the young cells since the unlysed cell population which contain young cells predominantly demonstrated a greater degree of incorporation than did the initial cell population.

c. The Chemical Nature of Antigen Specificity.

Extracts were made from chicken red blood cells by treatment with alcohol in an attempt to isolate and purify the "Hi" agglutinin. Fractions obtained by precipitation at cold temperatures, and from the dried alcoholic solution and residue were tested. The water soluble and insoluble components from the alcohol extracts did not possess inhibiting activity. However, the dry cell residue could still absorb agglutinin.

Using a similar technique human red blood cells of the A₁, B, O, and AB cell types were extracted and group specific material isolated. It appears that these substances are fairly active serologically, water soluble and nondialyzable. Isolation of a crude substance designated P₂ and a partially purified substance P₅ from each of the four blood types was achieved. The P₂ and P₅ substances are fairly stable, can survive heating at 100° C. for two hours but are completely inactivated by heating for two hours in 0.01 N HCl. Analyses of hydrolysates of the P₂ preparations reveal them to contain approximately 15% reducing sugar and 3% hexosamine. The P₅ fractions regardless of the type of blood cell from which they were derived yielded the same proportions of reducing substance, hexosamine, fucose, phosphorous and nitrogen confirming the studies of Koscielak and Zakrezewski, with A, B and AB bloods. The studies on human red blood cells was a dissertation presented as a partial requirement for a Master of Science degree at Georgetown University.

d. Model Studies on Antibody Specificity.

Studies were conducted to isolate and purify the agglutinins obtained from seed extracts of Phaseolus lunatus. Such extracts specifically agglutinate human A₁ and A₂ red blood cells. The use of chromatographic columns (DEAE diethyl amino ethyl cellulose) employing salt and/or pH gradient techniques established the presence of a minimum of four protein components which exhibit anti-A specific activity and two components lacking activity. Further rechromatographing, as well as analysis by vertical starch gel electrophoresis, resulted in the isolation of several dozen other components. Best results were obtained with carboxy-methyl cellulose and some preparations were obtained which were suitable for certain chemical analysis. Preliminary experiments conducted on several preparations show that all fractions so far tested contain hexosamine residues which vary in content from 2% to as much as 7% (mg hexosamine/mg protein). These results suggest the possibility that the effective charge on the protein molecule is largely determined by the hexosamine content. Thus, it may be visualized that these several protein moieties possess the property of a common specificity but fractionate as separate components on cellulose columns because they contain varying amounts of hexosamine complexed possibly to a common protein base.

Difficulties involved in the purification of anti-A agglutinins using conventional procedures prompted studies with human red cell stroma (ghosts) as an alternative method for purification. These absorption studies with ghosts became important for reasons other than purification per se when it was observed that antibodies could be fractionally eluted from ghosts at various temperatures. Subsequent experiments confirmed these observations.

The use of red blood cell membranes as antigen sources for the purification and study of antibodies by absorption and elution has not found wide application because of the difficulty of preparing them free of hemoglobin. A simple procedure has been developed in this laboratory which accomplishes the preparation of red blood cell membranes essentially free of contaminating hemoglobin. The method involves lysis of the red blood cells in a buffered solution with a surface active reagent, Triton X-100 and concentration of the cell membranes by centrifugation. Several washings are then required to remove hemoglobin. The "ghost" preparations under microscopic examination appear as translucent forms which retain a physical appearance similar to the parent whole red blood cell. No changes in antigenic specificity were observed in tests performed on them. Such red blood cell membranes free of hemoglobin makes possible a wide variety of applications for the purification and study of antibodies.

Experiments on absorption and elution of immune anti-A₁ antisera were carried out with A₁ and A₂ ghosts. It was possible to obtain fractions

of antibody at 56° C. which contained very little antibody capable of being eluted at 45° or 37° C. Similar purifications have also been obtained for fractions eluted at 37° C. Absorption and elution experiments have also been carried out with Phaseolus lunatus. In these experiments the agglutinin was washed free more readily following absorption suggesting that the energy required for thermal disruption of the bond with the A₂ antigen is considerably lower than that for the immune anti-A agglutinin.

These studies indicated that whereas the agglutinins and ghost preparations are relatively stable to heat, it is still possible to disrupt those bonds which are responsible for antigen-antibody combination. Further experiments were conducted using urea to test the possibility that hydrogen bonding plays a role in this combination. These preliminary studies show that hydrogen bonding effects cannot be excluded in antigen-antibody reactions.

B. Physiology

1. Avian Anatomy.

Many dissections, working drawings and descriptions have been completed for the various organ systems; however, most of the final art work remains to be done. Nearest to completion is the first volume of the series, which deals with the skin, feathers and other specialized structures of the surface of the body and is broken down into the following topics: (1) Topographic anatomy, (2) palpable underlying bony landmarks, (3) feather tracts of chicken, turkey, domestic duck, laboratory pigeon and quail, (4) feather types in each part of the body, (5) the pattern of feather muscles, (6) the details of muscle bundles between adjacent feathers, (7) sequence of development of many of the feathers of the body, (8) the time of molting of most of the feathers, (9) the distribution of nerves, arteries and veins from their central connections to all parts of the skin, feather muscles and dermal appendages, (10) the detailed structure of different parts of a feather, and in feathers from different regions of the body, (11) the microscopic study of the skin and all of its major structures, such as comb, wattles, ear lobes, claws, scales, etc., (12) the complex organization of a growing feather and finally (13) the distribution, growth, molting and significance of filoplumes.

A critical study of the gross lesions of turkeys suggestive of leukosis revealed that lymphoid and reticular cell hyperplasia resulted from subclinical blackhead infections or from the medication used to inhibit the progress of histomoniasis. Heretofore this hyperplasia or abnormal increase in the number of cells in turkeys had served as a potential basis for the condemnation of such birds at the time they were identified in processing plants.

This cooperative study on the part of the Agricultural Marketing Service and this Laboratory prompted a revision of the regulations used for inspection in processing plants, and the results have also been incorporated in the Poultry Inspector's Handbook. (AH e6-20, e6-26)

2. Composition of Uterine Tissue.

a. Ionic Composition of the Uterus in Cattle and Sheep.

Previous work had characterized the water and electrolyte composition of the uterine tissues of rabbits and cattle during various phases of the estrous cycle, during treatment with ovarian hormones and during pregnancy. These studies were completed and extended to sheep to ascertain the variation in electrolyte composition in this species. The endocrine state of ewes influenced the water and ion composition of the uterine endometrium and myometrium. Estrous and luteal-phase ewes had higher water concentrations in both tissues than did ovariectomized ewes. In the endometrium, extracellular water volumes and Na and Cl concentrations were highest in estrous, intermediate in luteal and lowest in ovariectomized ewes. In myometrium, variation in water content was similar to that in the endometrium. Extracellular water volume and Na content were lowest in estrous and highest in ovariectomized ewes, the opposite of values for the endometrium. Increasing age (varying from 1 to 10 years) was associated with a decrease in endometrial water content in estrous ewes ($r = -0.68$, $P < 0.01$) but not in luteal or ovariectomized ones. These associations suggest that the production or effect of ovarian hormones associated with estrous may be modified by age. The production or effect of ovarian hormones of the luteal phase are apparently not influenced by age. (AH h5-8)

b. Ionic Composition of the Rat Uterus During Tissue Growth.

The water and electrolyte changes in the rat uterus during the development of the artificial placenta, the deciduoma, were studied in detail to correlate temporal relationships during the early initial growth of decidual tissue (0, 24, 48, 72, and 96 hours after stimulation of the tissue). There is an immediate rapid H_2O imbibition, and a Na and Cl increase, changes which were maintained in all 3 groups during the first 48 hours. Thereafter, a different course of ionic events occurs in the rats with ovaries or progesterone treatment as compared to those without sources of ovarian hormones (ovariectomized). During the next 48 hours in the ovariectomized group the tissue recovers, i.e., the diameter, weight, Na, Cl and H_2O content decrease and approach control levels. In contrast, in the animals with progesterone (endogenous or exogenous), H_2O and ionic effects characteristic of tissue growth are superimposed upon the inflammatory phase

recovery effects. The rapid growth of new protoplasm masks any decrease in size of the uterus due to diminution of the inflammation. The Na and Cl increases are maintained as growth proceeds and the extracellular space is replaced with tissue. After an initial decrease, a marked increase in K, a cellular constituent is also noted. The high water concentration is maintained, but the fluid which was formerly extracellular shifts into the intracellular compartment. Thus, the new uterine tissue which develops during this period (48-96 hours) is of a different composition than that initially present, not only with regard to its proportion of endometrium and myometrium, but also with regard to hydration, extracellular and cellular constituents. The results of these investigations indicate that the early effects of inflammation are very similar to those of growth during the development of deciduoma. While these similarities would make it difficult to use one or more of these ionic growth parameters as an indicator of progesterone action and assay, they may indicate a common basis in the mechanism of hormone action, based upon permeability effects. Further work is in progress on the mediation of hormone action by histamine, an inflammatory agent, through alteration of membrane permeability. (AH h5-8)

c. Ionic Composition of the Rat Uterus and Placental Tissues During Pregnancy.

Previous work has been primarily concerned with characterization during the nonpregnant state. This study was undertaken to provide information on several uterine and placental constituents during pregnancy in the rat. Amounts of H_2O , Na, Cl, K, glycogen and histamine were determined at intervals of three days throughout gestation. Interdecidual uterine tissue became more hydrated during the latter half of pregnancy and the concentration of all the electrolytes studied also increased. This interdecidual tissue (largely myometrium) showed a steady rise in glycogen from day 9 to 21, there being a 6-fold increase in concentration at term. Histamine, on the other hand, decreased steadily in this tissue throughout pregnancy until at 21 days there was less than half of the initial value. Both the fetal and maternal portions of the placenta contained more H_2O than the interdecidual uterus, and the ions were generally lower. The maternal placental tissues showed a marked H_2O depletion on day 15 but partial recovery to former levels were evinced subsequently. A sharp drop in K level of the fetal placenta occurred at term. Both Cl and Na in the placental tissues tended to be low at day 15. Glycogen in the maternal placenta quadrupled during the last 8 days of pregnancy but during the same period the level of glycogen in the fetal placenta diminished to one-third of its earlier value. The histamine content of both placental tissues increased 2-3 times during the last half of pregnancy. (AH h5-)

d. Uterine Glycogen During Growth of Decidual Tissue.

The levels of glycogen, the principal storage form of carbohydrate energy, have been followed during the development of the artificial placenta, the deciduoma, in the rat. Previous work in other laboratories on uterine glycogen in rats indicated that estrogens cause glycogen deposition and this occurs only in the myometrium, while progesterone exhibits no effect. In this study intact as well as progesterone treated rats were utilized. With the development of deciduoma, the glycogen content of whole uterus increased from 68 to 125 mg/100g Wet Weight (W.W.). There was no change in myometrial glycogen, 74 mg/100g W.W. with no deciduoma and 73 mg/100g W.W. in decidual myometrium. Endometrial glycogen content of decidual tissue was 221 mg/100g W.W. Since myometrial glycogen was constant, the increases observed in the decidual tissue of whole uteri must be due to either an increase in the amount of endometrium and/or an increase in the concentration of glycogen in the endometrium. As the deciduoma developed the proportion of endometrium increased from 9% in the uninjured horn to 34% in the injured horn. Thus, an increase in the amount of endometrium contributes to the increase in the glycogen concentration. These results demonstrate that progesterone is responsible for the glycogen increase by stimulating the growth of endometrium, a glycogen-rich tissue. Since no endometrial tissue could be obtained from horns without decidual development, it was not possible to determine whether progesterone had any effect on glycogen deposition. (AH h5-8)

e. Uterine Histamine During Growth of Decidual Tissue.

Histamine, a constituent of almost all animal tissues, has recently been suggested as having a role in the progesterone controlled process of decidua formation. The level of histamine was, therefore, followed during the development of deciduoma, the artificial maternal placenta in the rat. In the process of inducing deciduoma, a stimulus acting upon a uterus in the proper hormonal state causes decidual tissue growth. A wide variety of stimuli, mechanical, electrical and chemical, are effective in acting as the inducing agent. A factor common to all of these inducing agents is tissue damage and histamine has long been known to be a substance associated with tissue injury. While its exact physiological role is difficult to assess, it seems clear that histamine is liberated at times of injury to tissues. The experimental technique utilized in these experiments involved the application of a scratch with a barbed needle to the surface of the endometrium of one uterine horn while the uninjured horn served as a control. As deciduoma developed, the injured horns with deciduoma exhibited a 50% decrease in histamine concentration when compared to the uninjured undeveloped uterine horns. In order to determine whether the scratching procedure or the growth of decidual tissue was responsible for the decrease in histamine concentration, the procedure was

carried out in ovariectomized rats without hormonal influences. Uterine horns which had been subjected to the scratching procedure were found to have a histamine concentration 100% higher than the uninjured horns. The response, therefore, to injury, inflammation and tissue repair was an increase in histamine concentration, while the decidual growth response resulted in a decrease in histamine concentration. (AH h5-8)

3. Endocrine Control of Uterine Defense Mechanisms.

A possible relationship exists between fertility and the uterine defensive mechanisms or their hormonal control. Investigations have been conducted on modes of endocrine control of the uterine defenses and possible species variation in factors related to the defenses.

Hormonal control of the acute inflammatory response was studied in sheep and cattle uteri. Twenty estrous and 20 luteal-phase ewes were inoculated in utero with Escherichia coli; infiltration of the endometrium by polymorphonuclear leukocytes, migration of leukocytes into the uterine lumen and clearance of E. coli from the uterine lumen occurred considerably slower in the luteal-phase ewes. This indicated that in sheep as well as in rabbits the presence of an active corpus luteum delayed the acute inflammatory response in the uterus. The association between leukocytic influx and bactericidal activity was significantly greater in luteal-phase than in estrous ewes, suggesting that extra-leukocytic defensive mechanisms may have been active in estrous ewes.

Thirty heifers were also inoculated in utero with E. coli. Both the leukocytic response and bactericidal activity were slower in luteal-phase heifers than in estrous or ovariectomized ones, indicating that progesterin inhibits the acute inflammatory response in uteri of this species also.

Comparisons among cattle, sheep and rabbits showed that leukocytic emigration was considerably slower in ewes than in heifers and rabbits. The physiological basis for this species variation is not known.

In all 3 species studied--rabbits, sheep and cattle--ovarian hormones influenced the uterine defenses primarily through an effect on the intensity of leukocytic emigration. This suggested an effect of the hormones on the vascular response to inflammatory stimuli. Heparin was given intravenously to rabbits at frequent intervals during acute inflammatory responses in the uterus. This anticoagulant did not delay leukocytic emigration or decrease the difference in leukocytic response between estrous and pseudopregnant rabbits. These results failed to suggest that intravenous fibrin formation was a factor in leukocytic diapedesis or that hormonal control of the leukocytic response was effected through such a mechanism.

Vascular permeability was also investigated as a possible means of endocrine control of the uterine defenses. Permeability of vascular endothelium was measured by the intensity of staining of extravascular uterine tissues after the intravenous injection of trypan blue dye. In uninoculated rabbits, estrogen maintained uterine vascular permeability at a high level; progesterin maintained it at a relatively low level, but slightly higher than in ovariectomized rabbits. After experimental uterine inoculation, vascular permeability increased in uteri of estrous and ovariectomized rabbits. Progesterin prevented this increase in pseudopregnant rabbits.

A highly significant relationship was found between leukocytic emigration and uterine vascular permeability during the acute inflammatory response. Both vascular permeability and intensity of leukocytic emigration were greatest in estrous rabbits, intermediate in ovariectomized rabbits and least in pseudopregnant ones. These relationships suggest that ovarian hormones control the uterine defense mechanisms in large part by controlling uterine vascular permeability. The most marked effect was exerted by progesterin, which almost completely prevented the vascular response to inflammation. This effect of progesterin is likely related to its functions in the maintenance of pregnancy.

Glucocorticoids have been reported to inhibit the ability of the vascular system to respond to vasoactive substances and to inhibit the early leukocytic response to skin infections. The effect of such hormones was investigated in experimentally-infected uteri of estrogen-influenced rabbits. Neither cortisone nor 9-fluoroprednisolone inhibited the leukocytic response or bactericidal activity. These glucocorticoids also had little effect on uterine vascular permeability. However, the injected hormones exerted a marked systemic effect; the normal increase in skin vascular permeability in response to intradermal injection of histamine or cell-free inflammatory exudates was almost completely inhibited. These results suggest that glucocorticoids have little effect in the estrogen-influenced uterus. (AH h5-8)

4. Uterine Defense Mechanisms Against Sperm.

Earlier work has shown that bull sperm are disposed of faster in estrual rabbits than are rabbit sperm. The present studies were undertaken in an attempt to clarify this difference and also to gain more insight into the uterine defense mechanisms. Dead bull sperm were removed more rapidly than live bull sperm in both estrual and luteal rabbits with the estrual rabbits disposing of both live and dead sperm more rapidly than the luteal. With dead and live rabbit sperm, no differences in rate of disposal were observed. The luteal rabbit disposed of both live and dead rabbit sperm quicker than the

estruual animal. Leukocyte numbers did not vary significantly in any of the groups studied. The ability of uterine cell-free exudates to remove sperm was determined. When sperm were suspended in exudates from estrual rabbits previously inoculated with sperm, bull sperm again were disposed of more rapidly than rabbit sperm. Prior inoculations with rabbit sperm produced more active exudates. Leukocyte numbers were not significantly affected by exudate type. (AH h5-6, cooperative with the University of Wisconsin)

5. Effect of Sperm Antibodies or Red Cell Antigens on Reproduction.

Previous work had shown that antibodies coming into direct contact with sperm can affect both fertilization success and embryo survival. These factors were investigated by inseminating 6 heifers with bull semen diluted with antiserum from a heifer immunized against the sperm of the bull. Five heifers returned to heat 21 days after breeding while the sixth had a degenerating embryo 42 days post breeding. Four other heifers were bred with semen diluted with either the "normal" serum of the heifer (obtained before immunization) or a modified Krebs solution. Normal appearing embryos were found in these heifers. A similar experiment in which 6 heifers were bred with bull semen diluted with antiserum from a rabbit immunized against the bull sperm showed similar results, all 6 returning to heat 19-29 days post breeding. When bred with "normal" rabbit serum, fertilized ovas were recovered. When the semen diluted with rabbit anti-bull sperm serum was treated with the sperm cells or the red blood cells (rbc) of the bull, absorption of the antiserum occurred with the sperm cells as evidenced by fertilized ova after use of the absorbed semen. The rbc were ineffectual in causing absorption of the antisperm serum and no fertilization occurred. Additional experiments demonstrated that rbc antiserum did not affect the capacity of semen to fertilize ova in cattle.

Similar results were obtained in rabbits, fertilized ova being recovered from rabbits bred with semen diluted with "normal" serum, sperm-absorbed antiserum and anti-rbc sera. Diluting the semen with antisperm serum or rbc absorbed antisperm serum prevented fertilization in all rabbits bred with these diluents.

The evidence from both the heifer and the rabbit work indicates that an antifertility factor exists in antisperm sera and not in normal sera. This factor prevents fertilization of ova when these sera are mixed with semen prior to insemination. The work also indicates a lack of cross reactivity between the antigens and antisera of sperm and red blood cells since (1) the antifertility effect can be removed from the antisperm sera by absorption with the sperm and not with the rbc's and (2) antisera against rbc's do not affect the ability of sperm to fertilize ova.

Studies were also undertaken at Beltsville to ascertain whether the fertilized ovum and the young embryo can be affected by bovine anti-sera against sperm and red blood cells (rbc) of rabbits. Ninety-five female rabbits have been studied to ascertain the effect of antisperm antibodies on development of fertilized ova. Ova were transferred from one female to another in normal serum (NS) or immune serum (IS). The NS was obtained from a heifer before she was given any injections. The IS was obtained from her following several injections of rabbit semen. The fertilized ova were treated directly with the IS to determine if the antibodies could exert their effect when used in this manner as compared to treating the sperm directly. Also, since considerable variability was noted among the females in previous work where sperm was treated, the NS and IS were tested within females. Ova treated with NS were transferred into one uterine horn and ova treated with IS into the other horn of the same female. Donor rabbits were killed 24 to 30 hours after breeding and their oviducts flushed with the appropriate serum. The ova in serum were then incubated at 37° C. until being transplanted into the oviduct of recipients. Thirty recipients were killed late in gestation to determine survival. Of 136 fertilized ova transplanted in NS, 31 (22.8%) survived as live fetuses while 37 of 127 ova (29.1%) transplanted in IS survived. Thus it appears that the antibodies did not affect survival. There was a positive relationship between the length of time the ova were incubated and the percent survival in both treatment groups. Eleven recipients received ova less than one hour from the time of the death of the donor. In these, 26 of 50 NS ova survived (52%) compared with 25 of 39 IS ova (64%). In studies at Wisconsin with the anti-rbc serum, 49% of the treated ova developed compared to 62% of the normal serum. When antisperm serum was injected into the uterine lumen containing 9-day embryos, only 14% survived to 28 days while 50% survived with normal serum injections. Forty-six percent and 30% of the embryos continued to develop after anti-rbc serum or normal serum injections. The similar results obtained at Beltsville and Wisconsin form the basis of a joint publication in press. (AH gl-7 and AH h5-6, cooperative with the University of Wisconsin)

6. Composition of Mammary Gland Tissue.

The composition of mammary gland tissue and changes during lactation, pregnancy, various endocrine states or in abnormal or disease conditions is not known. There has been little previous work in this area and studies were initiated on sheep mammary gland tissue to determine the normal composition in estrous and luteal sheep and in ovariectomized ewes. The changes occurring after experimental inoculation with bacteria were also determined. (AH h5-1)

a. Chemical Composition.

Initial studies were conducted with nonlactating and lactating sheep. The experimental induction of mastitis was undertaken using an exogenous source of bacterial infection, E. coli. Chemical changes in the mammary glands during the course of inflammation in differing hormonal conditions were followed since previous studies on uterine infection have indicated a difference in defense during either estrous or luteal states. Intramammary inoculations with E. coli were made on one-half of the udder while the other half served as a control. The fat content of mammary gland tissue is high and must be determined in order to express the analytical results in terms of concentrations per unit of fat-free tissue. Unless this is done it is impossible to correlate values from different animals or even different samples from the same mammary gland. When results were expressed in terms of fat-free tissue, there was a great uniformity with respect to water content and to the concentration of the inorganic and organic constituents in replicate samples.

Two and one-half to four hours after the experimental induction of mastitis by bacterial inoculation the treated udder half showed a great increase in size, being 2 times the weight and volume of the control half. This inflammatory response resulted in a dilution of the adipose tissue from about 20% of the total weight in control to about 10% in mastitic glands. Examination of the chemical composition of the mammary gland (water, fat, Na, K, Cl, glycogen, and histamine) during mastitis infection revealed changes in several of the constituents. The water content increased markedly, from about 81% to 87%. The response was somewhat greater in glands under estrous control than in glands from animals in the luteal phase of the estrous cycle. Na and Cl showed a large increase and K a decrease, indicating that the fluid increase probably occurs primarily in the extracellular compartment and is undoubtedly an inflammatory edema. A difference in the change in K content was noted between estrous and luteal glands, suggesting that the estrous gland response involved cellular elements to a greater extent than the luteal response. Whether this was due to mammary tissue cellular elements or to defense cellular elements such as leukocytes, has not yet been determined. Estimates of cell numbers and sizes during mastitis by means of DNA and RNA determinations and histological examination will disclose to what extent cell growth, hypertrophy and hyperplasia are involved in the inflammatory tissue changes. Histamine concentration of the inoculated glands decreased, indicating a mobilization and liberation of this substance which is involved in injury and damage in tissues. The most dramatic changes were observed in glycogen concentration between control and infected glands. Glycogen content increased 3 to 4 fold in the inoculated glands, with the estrous glands showing a larger increase. The results of these preliminary experiments indicate a correlation between the glycogen response and leukocytic invasion. Further work is in progress

to characterize this difference in the defense mechanism in mastitis produced by differing hormonal conditions.

b. Histopathology of Experimental Mastitis in the Ewe.

Mammary gland growth and development is controlled largely by ovarian hormones. An inhibitory effect by progesterone on mammary gland defenses during the initial phases of the inflammatory response might have a marked effect on the severity of mastitis and on the proportion of bacterial challenges to the mammary gland which result in clinical mastitis.

An investigation was conducted to determine the effect of ovarian status on the acute inflammatory response of the mammary gland. Non-lactating ewes in the breeding season were used as experimental animals; it was considered likely that an effect of ovarian hormones on mammary gland defenses could be detected in such animals. Mammary glands of paired estrous and luteal-phase ewes were inoculated with Escherichia coli and glands of estrous, luteal-phase and ovariectomized ewes were inoculated with Staphylococcus aureus.

Inflammation developed rapidly, with severe edema and lymphatic dilatation occurring within 2 hours. By this time the weight of inoculated glands had increased up to 4 times that of uninoculated glands. The leukocytic response occurred rapidly. By 3 to 4 hours the glands were heavily infiltrated by polymorphonuclear leukocytes; interstitial tissue was infiltrated diffusely and most ducts were filled with leukocytes. Microscopic examination and culture of gland cistern and ductular fluids indicated that bacteria were cleared rapidly from the gland as the inflammatory response developed. Phagocytosis by macrophages and microphages occurred widely in the inoculated glands, suggesting that neutrophilic leukocytes were major defensive agents against the introduced bacteria.

There were no consistent differences between estrous and luteal-phase ewes in the rate of development of the inflammatory response, thus indicating that progesterone probably had little if any effect on development of the acute inflammatory response in the mammary gland. The intensity of the initial leukocytic response was somewhat greater in estrous and luteal-phase ewes than in ovariectomized ones, indicating that ovarian hormones promote conditions in the mammary gland favorable to an intense inflammatory response.

Infiltration of the supramammary lymph nodes by neutrophilic leukocytes progressed rapidly. This neutrophilic infiltration indicated that products of inflammation, probably including bacterial toxins and small numbers of bacteria, entered the lymphatic system and reach the supramammary lymph nodes rapidly. This may be associated with the

finding by others of a rapid antibody production following infusion of antigen into the mammary gland. The supramammary lymph nodes of these ewes generally contained large numbers of plasma cells.

C. Nutrition

1. Metabolic Role of B₁₂

Previous work at Beltsville has shown that vitamin B₁₂ functioned in the metabolic disposal of the carbon moieties of four amino acids comprising the casein protein, namely: serine, valine, isoleucine and threonine. Additional studies implicated propionic acid formed in the catabolism of valine, isoleucine and probably threonine. Further investigations demonstrated that in the dominant pathway of catabolism of propionic acid in animals, in which this fatty acid is carboxylated to methylmalonic acid, B₁₂ is involved in the isomerization of the latter acid to succinic acid which is then metabolized in the citric acid cycle.

Propionic acid and also certain branched-chain and odd-carbon straight chain fatty acids which give rise to propionic acid in animal metabolism are normally formed in the rumen by microbial action on the feed and serve as an important source of energy for the host animal. Experiments with rats were carried out to study the relation of vitamin B₁₂ to the metabolism of these and other rumen acids. Various volatile fatty acids and related acids were fed to B₁₂-deficient and B₁₂-supplemented rats in a basal ration containing a normal level of protein, and the effects on growth and survival were followed.

Formic acid, fed as formate, brought about a significant growth depression in B₁₂-deficient animals and caused some deaths. With a supplement of the vitamin, no deaths occurred and growth remained normal. These results indicate that vitamin B₁₂ is concerned in the animal metabolism of formate.

In the absence of vitamin B₁₂, the even-carbon straight-chain rumen fatty acids, acetic and butyric, brought about no further lowering of the growth rate and caused no deaths among the animals. In contrast, propionic acid and the odd-carbon straight-chain n-valeric acid, which produces propionic acid in the course of its degradation in the animal body, caused significant depression in growth and led to death among the animals. Supplementing the rations containing the latter two acids with B₁₂ prevented deaths and allowed normal growth.

The branched chain acids--isobutyric and 2-methylbutyric--produced significant lowering of the growth rate in the absence of B₁₂ and deaths occurred among the animals fed the latter acid. When a supplement of B₁₂ was given, no deaths occurred and growth was not significantly different from normal with either acid. On the other hand,

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Department of Agriculture and cooperating agencies, a
summary of current program and preliminary report of
progress.

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the branched-chain isovaleric acid caused no further growth depression in B₁₂ deficiency. These results thus show that isobutyric acid and 2-methylbutyric acid, which are present in and are absorbed from the rumen, require B₁₂ for their catabolism.

Lactic acid, although not ordinarily but under some conditions present in the rumen, and pyruvic acid caused no further growth depression in the absence of B₁₂. Thus, cobalt in the form of B₁₂ is undoubtedly essential for the catabolism of certain rumen acids. (AH h4-3)

2. Synthesis of B₁₂ in the Rumen.

Biosynthesis by the rumen flora in the presence of cobalt is the sole or predominant source of B₁₂ for the ruminant. B₁₂ analogues, which may be important in normal maintenance of the flora themselves, are likewise thus synthesized. A survey has been conducted of the various functional microorganisms present in the rumen to determine which strains and species among them synthesize B₁₂ or B₁₂ analogues. The tests were made on pure cultures of a number of species of functional rumen organisms of the cow. All of these cultures were grown in essentially the same medium, which included 20% clarified rumen fluid, in an attempt to approximate conditions prevailing in the rumen. The assay method used for screening the organisms was the E. coli mutant 113-3 plate test.

Forty-eight strains, representing twenty-one different species, of rumen organisms were tested. These included representatives of most of the important genera isolated from the rumen of the cow. Only eight of the strains, representing four different species, synthesized an appreciable amount of B₁₂ or its analogues.

The active samples were also assayed by the E. coli tube test and with Ochromonas malhamensis. The latter test reflects true animal activity, while a comparison between the E. coli plate and tube tests gives some indication of the particular analogues being synthesized. The assays with Ochromonas indicated that the pure cultures in most cases did not produce as high a percentage of B₁₂ per se compared to the analogues as has been found in rumen fluid. While clarified rumen fluid was added to the culture medium to simulate as nearly as possible the conditions prevailing in the rumen, it is obvious that the conditions were not exactly the same where the various species of microorganisms grow symbiotically. However, it represents probably about as close an approach as can be made under pure culture conditions.

Studies to determine more exactly the particular B₁₂ analogues present in these cultures have been initiated, using the techniques of ionophoresis and chromatography combined with bioautography. Such tests have now been carried out on the Selenomonas strains. Two different analogues were observed, with almost all of these cultures

but their relative intensities varied from culture to culture. Thus, certain definite strains of rumen bacteria synthesize B₁₂ in the rumen. (AH h4-4)

3. Unidentified Nutrients.

Investigations on the still unidentified nutrients occurring in foods and feeds have been continued. For these studies, rats were used whose requirements for dietary essentials were increased by feeding them thyroprotein and thus making them hyperthyroid. The purified control ration fed to these rats contained all known nutrients in amounts required by the hyperthyroid rat. In addition, it contained cholesterol, which was found to stimulate the growth of such rats, and sulfasuxidine, which was found of value under test conditions in increasing the magnitude of the differences in weight gain between unsupplemented rats and those fed sources of unidentified nutrients. Differences in growth of 25 to 50 grams per rat in three weeks have been observed.

Experiments were carried out to throw light on the mode of action of sulfasuxidine in these tests. Two other sulfa drugs were tried for purposes of comparison--sulfathiazole and sulfathalidine. Sulfathiazole is absorbed from the digestive tract so rapidly that it seems to have little effect on the flora of the tract. Sulfathalidine, on the other hand, is so little absorbed that its action seems to be confined almost if not entirely to the intestinal flora. Sulfasuxidine is likewise absorbed only to a limited extent but seems to be more soluble than sulfathalidine. In these tests, the results obtained with sulfathiazole resembled those obtained with sulfasuxidine more nearly than those obtained with sulfathalidine. Thus, these experiments would seem to cast doubt upon the theory that the action of sulfasuxidine in these tests may be to prevent synthesis of some unidentified nutrient in the intestinal tract and suggest, rather, some systemic effect.

Storage of unidentified nutrients in the livers of rats and depletion under conditions where demand is increased by the feeding of thyroactive rations has been studied. Livers were collected from rats fed a thyroactive ration and compared in potency with those taken from rats fed a stock ration. As measured by the growth response of hyperthyroid test rats, the livers obtained from stock rats were significantly more potent in unidentified nutrients than those secured from hyperthyroid rats. These results indicate that the stress imposed upon rats by feeding them thyroactive rations tend to deplete their livers of their natural stores of these unidentified growth factors and fit in with the concept of them as "vitamin-like" in nature. Thus, the evidence continues to mount that there are still some unidentified vitamin factors despite the fact that evidence for their presence and their separation is proving to be difficult.

Tests of several lots of fishmeal for relative activity for unidentified nutrients indicated that they varied considerably in potency. The reasons for this variation are not clear, but they may be related to time and conditions of storage after processing.

Attempts at fractionating the active material from fishmeal have been made. Extraction with organic solvents (diethyl ether followed by acetone and the absolute alcohol) yielded evidence suggesting that some of the active material might be associated with a lipoprotein fraction. When the fishmeal was first extracted with alcohol so as to denature the protein, these organic solvents were no longer able to remove active material. The fishmeal residue still contained all or almost all the activity as well as 83% of the solids of the original fishmeal. Further purification was accomplished by hydrolysis with H_2SO_4 treatment with alcohol, neutralization, extraction with acetone and ether and absorption on Norit. The solids on the Norit which appeared to contain much of the activity consisted of about 0.6% of the weight of the original fishmeal and represented an intake of about ten milligrams per test rat per day. In other experiments, treatment with various concentrations of alcohol, acetone or an alkaline 70% alcohol solution failed to remove the activity from Norit. (AH h4 1)

4. Basic Research on Ruminant Metabolism.

Dairy goats have been used in basic research on ruminant metabolism in cooperation with the University of Maryland. Studies on the metabolism of acetate and palmitate in the ruminant liver were carried out with goat livers in vitro in an artificial heart lung perfusion system using acetate $1\ C^{14}$ and palmitate $1\ C^{14}$. These studies confirmed the finding that large amounts of acetate pass through the liver without being metabolized and also demonstrated the utilization of acetate for liver oxidations and synthesis of various lipids. Palmitate has been shown to be oxidized by the liver and a rapid exchange between liver and blood long chain fatty acids has been demonstrated. Several lactating goats have been fed radioactive S^{35} to label the milk proteins and sulfolipids. This labeled milk has thus been used in studies on milk chemistry. (AH b4 3)

5. The Metabolism of Zinc by Rabbits.

Research was undertaken at Madrid, Spain, under PL 480 to study the metabolism and excretion of zinc in animals. Zinc 65 chloride was quickly absorbed after injection into the peritoneum of rabbits. Organs which take up and lose zinc quite rapidly include the liver, spleen, pancreas, reproductive organs and bowels. Organs like the brain and muscle reach the highest level of concentration after a relatively long time and they lose the zinc quite slowly also. Accumulation of zinc takes place principally in the bones. Excretion is

mainly through the feces. The excretion through the urine in male rabbits is practically nil. (E-25-AH-4)

C. Rumen Function

This work primarily relates to studies on rumen microbiology and metabolism in cattle. It is largely basic in nature but is also pertinent to work on the nutritional efficiency of cattle. (AH h2-3)

1. Amino Acid Catabolizing Bacteria.

Studies on the bacteria responsible for the catabolism of amino acids to NH_3 and volatile fatty acids in the rumen indicated that Bacteroides rumenicola was important. In addition to catabolizing some other amino acids it catabolizes branched-chain amino acids such as leucine with the production of branched-chain volatile acids such as isovaleric acid and NH_3 required for growth of many other rumen bacteria including cellulose digesters. Thus, B. rumenicola is an important component in microbial interactions affecting protein synthesis and breakdown and cellulose digestion in the rumen.

2. Growth Requirements of Rumen Bacteria.

Studies on many strains of previously identified bacteria known to be functioning in the rumen of cattle fed a wide variety of rations and on strains freshly isolated from the rumen revealed that most strains of most species can now be grown in defined media or in media in which vitamin-free casein hydrolysate is the only ingredient not defined (86% of strains in one study). Relatively few of these bacteria require higher fatty acids, nucleic acid derivatives of many other growth factors not included in the media. Almost all of the bacteria require one or more B-vitamins.

Nitrogen sources for growth are usually very simple in that NH_3 serves this purpose in most strains (80% in one study) of most species. Some of the latter are versatile in that they can utilize either NH_3 or amino acid mixtures as nitrogen source. However, some species (six species and 26% of total strains in one study) require NH_3 for growth and fixed amounts approximating the amount of protein nitrogen produced even when large amounts of organic nitrogen (amino acids and peptides) are present in the growth medium. These same species and some others which do not require NH_3 , i.e., they can utilize exogenous amino acid nitrogen, utilize very little exogenous amino acid carbon (C^{14}) in protein synthesis. Thus, the rumen contains many organisms that have to synthesize most amino acid constituents of their protein from NH_3 and exogenous nonamino acid carbon. This suggests that the ability to efficiently utilize amino acids is of little survival value in the microhabitat of many rumen bacteria perhaps because little amino acid is available to them. No other heterotrophic organisms

are known which synthesize most of their cellular amino acids when exogenous amino acids are available. A small proportion of rumen bacteria require amino acids for growth (6% in one study) and about half of the species efficiently utilize exogenous amino acid carbon.

It was found that one or more of the volatile fatty acids, isovalerate, isobutyrate, 2-methylbutyrate and n-valerate are required for growth of many strains of rumen bacteria (20% in one study) and that these include several species other than the cellulolytic species previously reported.

3. The Function of Isovalerate in Rumen Bacteria.

Studies utilizing isovalerate-1 and 3 C¹⁴ and the cellulolytic bacterium Ruminococcus flavefaciens showed that the intact carbon skeleton was utilized for synthesis of leucine of cellular protein. Further studies showed that the carboxyl carbon of leucine synthesized from isovalerate was derived from CO₂, i.e., a CO₂-fixation reaction. This mechanism of leucine synthesis was not previously known. C¹⁴ studies with whole rumen contents indicate that this mechanism is important in leucine synthesis in the rumen and that a similar mechanism for synthesis of valine from isobutyrate and CO₂ is also functional.

It was also shown that isovalerate and isobutyrate serve as sources of the branched-chain moiety in biosynthesis of higher branched-chain fatty acids and aldehydes in pure cultures of cellulolytic bacteria. Much of the higher fatty acid and aldehydes was found in phospholipids. This is the first report of these higher aldehydes in bacteria and indicates that plasmalogens are of significance in ruminal bacteria. The functions of these acids and aldehydes in the cell are unknown. Attempts to replace the isovalerate growth requirement of R. flavefaciens with lipids or lipid fractions extracted from R. flavefaciens cells were unsuccessful.

The results suggest that branched-chain volatile acids are required for growth of certain rumen bacteria because of a limited ability or inability to synthesize the isopropyl moiety found in branched-chain amino acids and in lipid and a limited ability to incorporate exogenous amino acids.

4. The Nature and Importance of Rumen Microbial Lipids.

In cooperation with workers of the University of Maryland, branched-chain and odd carbon higher fatty acids and aldehydes similar to those of the cellulolytic bacteria were identified as major components of the bacterial fraction of rumen contents. The quantity and character of those microbial lipids are interpreted as indicating that

significant quantities of ruminant fatty acids including milk fat acids and aldehydes originate from rumen microbial synthesis of long chain acids and aldehydes from volatile fatty acids.

5. Hemin Metabolism in the Rumen.

In studies on the nutrition of Bacteroides ruminicola, an important rumen bacterium, it was found that hemin replaced the "rumen fluid" factor requirement of subspecies ruminicola which usually accounts for about 80% of strains of B. ruminicola isolated from rumens of cattle. Studies indicated that this organism utilizes a wider range of tetrapyrroles than any heme-requiring organism previously studied but that many compounds which replace heme requirements in some microorganisms are inactive. Compounds which replaced heme in iron containing medium included catalase, peroxidase and hemoglobin, proto-, hemato-, meso-, and deuteroporphyrin, zinc and manganese protoheme, and uro and coproporphyrinogen. Compounds which would not replace hemin included cytochrome C, bilirubin, chlorophyll, citrate, ethylenediaminetetraacetic acid, ferrichrome, coprogen, terregens factor, delta-aminolevulinic acid, porphobilinogen, coproporphyrin, uroporphyrin and copper protoheme.

Heme independent B. ruminicola subsp. brevis excretes porphyrin, probably mainly coproporphyrinogen, into the medium during growth and washed cells produce porphobilinogen and porphyrin from delta-aminolevulinic acid. Subspecies ruminicola does not have these abilities. The results indicate that B. ruminicola synthesizes porphyrins via mechanisms similar to those found in other organisms and subspecies ruminicola lacks one or more of the enzymes in the pathway between succinate plus glycine and uroporphyrinogen.

Studies on cellular heme compounds of both subspecies indicated that they contained a cytochrome similar to cytochrome o but of unknown function and a cytochrome of the b type involved in an electron transport system, also containing flavoprotein, by which DPNH generated by glycolysis causes the reduction of fumarate to succinate, the major fermentation product of this organism and an important intermediate in propionate production in the rumen. No other hemoproteins were detected and all of the heme extracted from cells appeared to be protoheme. This is the first indication that cytochromes are involved in energy metabolism in the rumen or in any strictly anaerobic, carbohydrate fermenting organism. Also, the studies seem to give at least one reason for the known iron requirement for protein synthesis, i.e., bacteria growth, in the rumen. Since the heme independent subspecies has the same functions as the heme requiring subspecies and produces porphyrin utilized by the latter, it is doubtful that absence of porphyrins in ruminant rations would reduce the efficiency of the rumen fermentation. This work was done in cooperation with D. C. White and S. Granick of the Rockefeller Institute, New York.

By following oxidation and reduction of the cytochrome b and flavo-protein in cells of Bacteroides ruminicola, evidence was obtained that succinate was produced from carbohydrate via CO₂ fixation to pyruvate one related compound, produced by glycolysis, and reduction of the resulting oxalacetate to succinate via malate and fumarate.

6. An Improved Culture Medium for Rumen Bacteria.

A new rumen fluid medium has been developed for the nonselective culture, counting and isolation of ruminal bacteria. This medium supports the growth of about double the colonies of the old medium, colonies are easier to count and many more can be counted per tube. That counts were doubled but similar proportions of species were isolated as compared to the old medium indicates that some of the discrepancy between culture counts and direct microscopic counts of rumen contents is due to failure of some cells of species cultured to grow in artificial media rather than failure to grow some species.

7. Affect of Animal Management on Rumen Bacterial Counts.

Using the new medium, it was shown that total b viable counts of rumen bacteria vary significantly in relation to the ration consumed, time of sampling relative to time of feeding and watering, the number of times a day the animal was fed, the position in the reticulo-rumen samples and in relation to unknown factors. It is evident that these viable counts are an excellent tool for use in obtaining a better understanding of the kinetics of the ruminal fermentation.

8. Digestion of Highly Resistant Cellulose Fibers.

In studies with a visiting scientist from Rowett Research Institute in Scotland, it was shown that strains of cellulolytic bacteria previously isolated and characterized in this laboratory and shown to digest partly degraded cellulose, i.e., finely ground cotton or filter paper, could also digest highly resistant, native cellulose from cotton fibers. Such data adds to an understanding of the function of these bacteria in digesting cellulose and shows that they are truly cellulolytic. (AH h4-3 and is also reported in Area 1, B-2)

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Genetics

- Blair, Paul V. 1961. Quantitative consequences of mating systems and selection methods. Ph. D. Thesis. Purdue University
- Blair, P. V., Kyle, W. H., Anderson, V. L., and Bell, A. E. 1961. Quantitative consequences of mating systems and selection methods. Genetics, 46, p. 852.

- Scheinberg, S. L., and Reckel, R. P. 1961. The effect of estrogen on somatic variation of an erythrocyte agglutinin in chickens. *Nature*, 189, pp. 295-296.
- Scheinberg, S. L., and Reckel, R. P. 1961. Detection of red cell agglutinogens with lectins in chickens. *Poultry Science*, 40, pp. 689-698.
- Scheinberg, S. L., and Reckel, R. P. 1961. Somatic variation of red cell agglutinogens due to hormonal influence. *Poultry Science*, 40, pp. 796-807.
- Scheinberg, S. L., and Reckel, R. P. 1962. Studies on the "Hi" agglutinin in chickens. *Annals N. Y. Acad. Sciences*, 97, pp. 194-204.
- Wong, D., and Scheinberg, S. L. 1962. Purification of Phaseolus lunatus lectin. *The Immunogenetics Letter*, 1(4), p. 15.

Physiology

- Bitman, Joel, Wrenn, T. Randall, Cecil, Helene C., and Sykes, J. F. 1960. Water and electrolyte composition of decidual tissue of the rat uterus. *Acta Endocrinologica*, 34, p. 465.
- Brinsfield, T. H., Richter, H. F., and Hawk, H. W. 1962. Inhibition by ovarian hormones of the inflammatory response in the sheep uterus. *J. Dairy Sci.*, 45, p. 682. (Abstract)
- Broome, A. W., Winter, A. J., McNutt, S. H., and Casida, L. E. 1960. Variations in uterine response to experimental infection due to the hormonal state of the ovaries. II. The mobilization of leukocytes and their importance in uterine bactericidal activity. *Amer. Jour. Vet. Res.*, 21, p. 675.
- Cecil, H. C., Wrenn, T. R., Bitman, J., and Sykes, J. F. 1960. Water and electrolytes in rat deciduoma. *Fed. Proc.*, 19, p. 168.
- Cecil, H. C., Bitman, J., and Sykes, J. F. 1962. Glycogen in the decidual tissue of the rat uterus. *Fed. Proc.* 21, p. 208. (Abstract)
- Hawk, H. W., Turner, G. D., and Sykes, J. F. 1960. The effect of ovarian hormones on the uterine defense mechanism during the early stages of induced infection. *Am. J. Vet. Res.*, 21, p. 644.
- Hawk, H. W., Turner, G. D., and Sykes, J. F. 1960. The bactericidal properties of uteri and uterine exudates of rabbits with reduced leukocytic activity. *Am. J. Vet. Res.*, 21, p. 649.
- Hawk, H. W., Turner, G. D., and Sykes, J. F. 1960. Noncellular bactericidal factors in inflammatory exudates from the uterine and pleural cavities of estrous and pseudopregnant rabbits. *Am. J. Vet. Res.*, 21, p. 657.
- Hawk, H. W., Turner, G. D., and Sykes, J. F. 1960. Variations during the estrous cycle in the inflammatory response and bactericidal activity of the sheep uterus. *J. An. Sci.*, 19, p. 1325.
- Hawk, H. W., Turner, G. D., and Brinsfield, T. H. 1961. Water and ion concentrations in the sheep uterus. *J. An. Sci.*, 20, p. 972.
- Hawk, H. W., Turner, G. D., and Sykes, J. F. 1961. Variation in the inflammatory response and bactericidal activity of the sheep uterus during the estrous cycle. *Am. J. Vet. Res.*, 22, p. 689.

- Hawk, H. W., Turner, G. D., and Sykes, J. F. 1961. The leukotaxic properties of uterine exudates as related to the endocrine-controlled uterine defense mechanism. *Am. J. Vet. Res.*, 22, p. 1117.
- Hawk, H. W., Righter, H. F., and Brinsfield, T. H. 1962. Effect of ovarian status on the acute inflammatory response in the sheep mammary gland. *J. Dairy Sci.*, 45, p. 682. (Abstract)
- Inskeep, E. K., Oloufa, M. M., Pope, A. L., and Casida, L. E. 1961. Functional capabilities of experimentally induced corpora lutea in ewes. *J. An. Sci.*, 20, p. 974.
- Lucas, Alfred M., and Denington, Effie M. 1961. A brief discussion of normal and abnormal avian blood cells. *Avian Diseases*, 5, pp. 454-455.
- Lucas, Alfred M., and Denington, Effie M. 1961. A brief report of anatomy, histology, and reactivity of air sacs in the fowl. *Avian Diseases*, 5, pp. 460-461.
- Lucas, Alfred M., and Jamroz, Casimir. 1961. *Atlas of Avian Hematology*. USDA Agr. Monograph, 25, pp. 1-271.
- Winter, A. J., Simon, J., McNutt, S. H., and Casida, L. E. 1960. The effect of an induced state of leukopenia on the uterine bactericidal activity in rabbits. *Amer. Jour. Vet. Res.*, 21, p. 644.
- Winter, A. J., Broome, A. W., McNutt, S. H., and Casida, L. E. 1960. Variations in uterine response to experimental infection due to the hormonal state of the ovaries. I. The role of cervical drainage, leukocyte numbers and noncellular factors in uterine bactericidal activity. *Amer. Jour. Vet. Res.*, 21, p. 668.
- Wrenn, T. R., Cecil, H. C., and Bitman, J. 1962. Chemical composition of mammary gland during experimental mastitis in the ewe. *J. Dairy Sci.*, 45, p. 682. (Abstract)

Nutrition

- Dryden, L. P., Riedel, G. H., and Hartman, A. M. 1960. Unidentified nutrients required by the hyperthyroid rat. *J. Nutrition*, 70, pp. 547-560.
- Dryden, L. P. 1961. The production of vitamin B₁₂ and vitamin B₁₂ analogues by rumen microorganisms. Thesis accepted by the American University, Washington, D. C., (in partial fulfillment of the requirements for the degree of Master of Science.) (University Microfilms).
- Hartman, A. M., and Dryden, L. P. 1960. Relation of vitamin B₁₂ to metabolism of protein fed at high levels. Fifth International Congress on Nutrition. p. 61. (Abstract)
- Hartman, A. M., and Dryden, L. P. 1962. Vitamin B₁₂ and the metabolism of rumen fatty acids. *J. Dairy Sci.*, 45, p. 691. (Abstract)

Rumen Function

- Allison, M. J. 1961. Isovaleric and isobutyric acids as growth factors for Ruminococcus flavefaciens, with studies on the metabolic function of isovalerate. Ph. D. Thesis. University of Maryland.
- Allison, M. J., Bryant, M. P., and Doetsch, R. N. 1962. Studies on the metabolic function of branched-chain volatile fatty acids, growth factors for ruminococci. I. Incorporation of isovalerate into leucine. J. Bacteriol., 83, pp. 523-532.
- Allison, M. J., Bryant, M. P., Katz, I., and Keeney, M. 1962. Studies on the metabolic function of branched-chain volatile fatty acids. II. Biosynthesis of higher branched-chain fatty acids and aldehydes. J. Bacteriol., 83, pp. 1084-1093.
- Allison, M. J., Bryant, M. P., Keeney, M., and Katz, I. 1961. The metabolic fate of isovalerate, a growth factor for Ruminococcus flavefaciens. J. Dairy Sci., 44, p. 1203. (Abstract)
- Allison, M. J., and Katz, I. 1962. Branched-chain fatty acids of Bacillus subtilis. Bacteriol. Proc., p. 35. (Abstract)
- Bladen, H. A. 1962. Isolation and identification of rumen bacterial species which produce ammonia from a protein hydrolysate and factors concerning this production. Ph. D. Thesis. University of Maryland.
- Bladen, H. A., Bryant, M. P., and Doetsch, R. N. 1961. Production of isovaleric acid from leucine by Bacteroides rumenicola. Tech. Note J. Dairy Sci., 44, pp. 173-174.
- Bladen, H. A., Bryant, M. P., and Doetsch, R. N. 1961. A study of bacterial species from the rumen which produce ammonia from a protein hydrolysate. Appl. Microbiol., 9, pp. 175-180.
- Bryant, M. P. 1960. Some aspects of ruminal metabolism by pure culture studies of the indigenous flora. Proc. of 8th Int. Grassland Congress, pp. 518-522.
- Bryant, M. P. 1961. The nitrogen metabolism of pure cultures of ruminal bacteria. ARS 44-92, 15 pages.
- Bryant, M. P., Barrentine, B. F., Sykes, J. F., Robinson, I. M., Shawver, C. B., and Williams, L. W. 1960. Predominant bacteria in the rumen of cattle on bloat-provoking ladino clover pasture. J. Dairy Sci., 43, pp. 1435-1444.
- Bryant, M. P., and Robinson, I. M. 1960. The nutritional requirements of ruminal cellulolytic bacteria. J. Dairy Sci., 43, p. 876. (Abstract)
- Bryant, M. P., and Robinson, I. M. 1961. Some nutritional requirements of the genus Ruminococcus. Appl. Microbiol., 9, pp. 91-95.
- Bryant, M. P., and Robinson, I. M. 1961. Studies on the nitrogen requirements of some ruminal cellulolytic bacteria. Appl. Microbiol., 9, pp. 96-103.
- Bryant, M. P., and Robinson, I. M. 1961. An improved nonselective culture medium for ruminal bacteria and its use in determining diurnal variation in numbers of bacteria in the rumen. J. Dairy Sci., 44, pp. 1446-1456.

- Bryant, M. P., Robinson, I. M., and Lindahl, I. L. 1961. A note on the flora and fauna in the rumen of steers fed a feedlot bloat-provoking ration and the effect of penicillin. *Appl. Microbiol.*, 9, pp. 511-515.
- Bryant, M. P., and Small, N. 1960. Observations on the ruminal microorganisms of isolated and inoculated calves. *J. Dairy Sci.*, 43, pp. 654-668.
- Caldwell, D. R., White, D. C., and Bryant, M. P. 1962. Specificity of the heme requirement for growth of Bacteroides ruminicola--a ruminal saccharolytic bacterium. *J. Dairy Sci.*, 45, p. 690. (Abstract)
- Keeney, M., Katz, I., and Allison, M. J. 1962. On the probable origin of some milk fat acids in rumen microbial lipids. *J. Amer. Oil. Chem. Soc.*, 39, pp. 198-201.

MISCELLANEOUS INFECTIOUS AND NON-INFECTIOUS DISEASES OF ANIMALS
Animal Disease and Parasite Research Division, ARS

Problem. Included in this area of research are diseases such as vesicular stomatitis, which affects cattle, horses, swine, and man; poisoning by various plants, which differ in toxicity according to local conditions, and affect different species of animals in various ways; agricultural chemicals, such as herbicides and pesticides, which may produce poisoning in animals, especially if not properly used, and may also leave dangerous residues in the soil, feed, or animal body; tumors, including cancer, which affect all species of animals; bloat, a common, serious condition in cattle and sheep; and potential dangers of "fall-out" from nuclear testing or attack. Investigations of these diverse hazards to livestock and poultry require modern techniques as well as fundamental approaches through chemistry, pathology, physics, physiology, and other scientific disciplines. The problems are so complex, diverse, and numerous that it has been impossible to more than scratch the surface in probing for basic knowledge required for protection of the nation's livestock and poultry populations.

USDA PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, pathologists, physicists, and veterinarians engaged in both basic studies and the application of known principles to the solution of miscellaneous infectious and non-infectious diseases of animals. Research is being conducted at the designated locations.

The Federal scientific effort devoted to research in this area totals 18.0 professional man-years. This effort is divided among sub-headings as follows:

Incidence and Pathology of Tumors 1.0 at the National Animal Disease Laboratory, Ames, Iowa. A grant of P.L. 480 funds equivalent to \$51,383 has been placed with the Veterinary Faculty, Ankara University, Ankara, Turkey, on etiologic investigation of bovine urinary bladder tumors due to enzootic bovine hematuria in Turkey and its relation to bovine papilloma agent.

Vesicular Stomatitis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Components of Normal and Immune Serum 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Bloat in Ruminants 4.5 at the National Animal Disease Laboratory, Ames, Iowa, and through cooperative agreements with the California, Maryland, and Mississippi Agricultural Experiment Stations and with the New York State Veterinary College.

Preparedness for Diagnosis of Foreign Animal Diseases 2.5 at the Plum Island Animal Disease Laboratory, Plum Island, New York.

Toxicology and Pathology Related to Insecticides 2.5 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas, in cooperation with the Entomology Research Division.

Biochemical Effects of Agricultural Chemicals 0.9 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas, and through Cooperative Agreement with the Stephen F. Austin College at Nacogdoches, Texas.

Detoxication Mechanisms in Cattle and Sheep 0.5 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas.

Cytological Responses to Antiparasitic and Other Agricultural Chemicals 0.5 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas.

Poisoning by Plants 1.1 at the Logan, Utah, Field Station and through cooperative agreement with the Utah Agricultural Experiment Station and through informal cooperation with the U. S. Plant Soil and Nutrition Laboratory of Soil and Water Conservation Service, Ithaca, New York. A grant of P.L. 480 funds equivalent to \$56,746 has been placed with the Instituto Biologico, Sao Paulo, Brazil, on the study of plants of the State of Sao Paulo poisonous to domestic animals.

Toxicity of Herbicides and Herbicide-Treated Plants for Domestic Animals 1.0 at the Logan, Utah, field station with informal cooperation with the Utah Agricultural Experiment Station and the Crops Protection Branch of the Crops Research Division at Logan, Utah.

Alleviators and Diagnostic Tests for Plant Poisoning 1.0 at the Logan, Utah, field station through informal cooperation with the Utah State University, the Crops Research Division and the Forest Service.

The Susceptibility of Wild Animals to Foot-and-Mouth Disease 0.5 at the Plum Island Animal Disease Laboratory, Plum Island, New York.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 16.4 divided among subheadings as follows: Poisoning by Plants 3.1 at Arizona, South Dakota, Texas, Utah, Wyoming. Biochemical Effects of Agricultural Chemicals 3.0 in the North Central, Southern, and Western Regions. Bloat in Ruminants 6.3 at Iowa, Minnesota, and Wisconsin Agricultural Experiment Stations. Other Conditions-Pathology of Tumors, etc. 4.1 in all four regions.

Industry and other organizations are engaged substantially in research and development of agricultural parasiticides, herbicides, feed additives, insecticides, and pesticides that may be used in production of both feed and livestock, or may endanger livestock exposed to them through misuse, miscalculation, or accident. Aggregate expenditures by chemical and other companies in relation to these products probably are as great or greater than those in any other single agricultural market area. USDA, State stations, veterinary schools and colleges, and others are called upon from time to time for assistance or collaboration in evaluation or safety-testing of compounds that are potentially useful to the livestock industry. Samples of new, ostensibly useful and safe compounds are frequently proffered free of charge for comparative laboratory or field testing with other previously used materials. Such offers to USDA are accepted when testing is deemed both practicable and directly useful in the over-all program of research aimed at development of sound programs of prevention and control of disease of livestock and poultry. In its search for more effective compounds in the area of miscellaneous infectious and non-infectious diseases of animals, and in toxicological studies, it is estimated that industry devotes no less than 20 professional man-years per annum.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Incidence and Pathology of Tumors.

Work of ten years was closed on tumors at the Denver Animal Disease Laboratory during 1961. In 1962 the equipment, specimens, and that finished portion of the colored atlas of animal pathology with planned concise descriptions of 75 selected animal diseases was moved to the National Animal Disease Laboratory, Ames, Iowa, for the current work.

The results obtained so far during 1961 and 1962 under Public Law 480 with Turkey on investigations as to the cause of bovine urinary bladder tumors, associated with or due to enzootic hematuria, have indicated that spontaneous tumors of bovine urinary bladders produced papilloma in the skin of calves.

B. Components of Normal and Immune Serum.

In 1961 the following is a summary of the work done at the National Animal Disease Laboratory at Ames, Iowa.

The technique of separating high and low molecular weight agglutinins for Brucella by ultracentrifugation of serum layered over a density gradient sucrose solution (10 to 40 percent) was applied to 19 serum samples collected from 4 pregnant heifers in the interval of 15 to 63 days after exposure to virulent Br. abortus strain 2308. Within the period of 15 to 22 days after exposure, the serum agglutinins were predominately of high molecular weight (80 to 100 percent). The amounts of high molecular weight agglutins remained fairly constant in the period of 22 to 63 days after exposure. At about 30 days following exposure, significant levels of low molecular weight were evident, and by 63 days following exposure increased to the extent of constituting 70 to 94 percent of the total agglutinins.

Density gradient centrifugation studies were made on 80 serum samples collected from 20 heifers, 5 to 91 days after vaccination with Br.abortus strain 19. High molecular weight agglutinins constituted 86 to 100 percent of the agglutinins present in serums collected in the interval of 5 to 15 days post-vaccination. Relatively small amounts of low molecular weight agglutinins were found in serums collected from 8 animals in the interval of 17 to 91 days post-vaccination.

The heat inactivation test (65°C., 15 minutes) indicated a fairly close correlation between the percent heat labile agglutinins and percent high molecular weight agglutinins in the serums from the vaccinated animals. The titers obtained with the 56°C., 18-hour heat inactivation test of Hoerlein on serums from 16 heifers 5 to 10 days post-vaccination, were very similar to the titers obtained with the standard tube test.

In 1962 the M-2 glycoprotein of bovine plasma was prepared by the ammonium sulfate-carboxymethyl cellulose method (A. Bezkorovainy and D. G. Doherty, Archives Biochem. Biophys. 96, 491, 1962), and a detailed study of its molecular weight and thyroxine-binding properties was made. Molecular weight was determined by sedimentation-diffusion, sedimentation-viscosity-, and Archibald methods, and was found to be 48,000 at pH 7.0. The weight-average molecular weight increased when pH was decreased below pH 4.0. Thyroxine-binding properties of the M-2 glycoprotein are of considerable interest not only because the system can serve as a model for thyroxine-protein interaction studies, but also because the M-2 glycoprotein bears considerable similarities to the physiological thyroxine carrier of bovine blood. The M-2 glycoprotein did not bind thyroxine in its native form, however, "activation" could be brought about by either heating the protein at 80° for 10 min. or by subjecting it to pH 3 for one hour.

Free energy of binding of thyroxine by the M-2 glycoprotein after "activation" was found to increase linearly with decrease in pH from pH 10.4 to 8.6 and extrapolation to pH 7.4 yielded a $\Delta F = -7.5$ KCal/mole at 27° and -6.8 KCal/mole at 4°C with two binding sites per protein molecule. These binding energies are significant by being higher than those of bovine serum albumin.

C. Bloat in Ruminants.

This work is being done at the National Animal Disease Laboratory at Ames, Iowa, and under cooperative agreements with the California, Maryland, Mississippi, and Wisconsin Agricultural Experiment Stations and with the New York State Veterinary College at Cornell University. In 1961 there was also work done under cooperative agreement with the Minnesota Agricultural Experiment Station. Work is also under way in cooperation with the North Central Regional Project NC-27 on Chemistry and Physiology of Bloat, and through informal cooperation with the Animal Husbandry Research Division.

In 1961 the California Agricultural Experiment Station at Davis, in cooperation with the USDA, collected physiological differences between bloat susceptible and non-susceptible cattle for a third year, and demonstrated an association between bloat susceptibility and salivary secretion rate.

In 1962, at the California Agricultural Experiment Station, a study was made of the relationships between the nature of the diet and rumino-reticular motility. Rumino-reticular motility has been recorded in cattle fed freshly harvested alfalfa tops, Sudan hay, or finely ground, pelleted alfalfa. The frequency and amplitude of mixing and eructation contractions were greater during and following the feeding of alfalfa tops than during and following the feeding of Sudan hay. Pre-feeding motilities were similar. These results suggest that theories which explain bloat prevention by coarse roughages on increased rumino-reticular activity and efficiency of eructation require modification.

Feeding finely ground, pelleted alfalfa hay results in a marked reduction in the amplitude of rumen contractions in comparison with those recorded on long-stem alfalfa hay. This reduction occurs within a few days after the initiation of pellet feeding; motility remains at a relatively low level for as long as 3 months. Recovery of "normal" motility pattern occurs within a week after resuming feeding long hay. A partial explanation of the mild and moderate bloat associated with pelleted and finely ground feeds seems to be a reduction in the strength and efficiency of eructation.

In 1961, in cooperation with the University of Maryland, College Park, a study was made of the pharmacological effects of various sympathomimetic and parasympathomimetic drugs on the eructation mechanism of sheep subjected to various bloat-producing feeding situations. Intramuscular injections of adrenalin and atropine produced failure of eructation and bloat in sheep, goats, and cattle. Oral administration of L-tyrosine also produced bloat, presumably because certain rumen bacteria decarboxylate tyrosine to tyramine, which is toxic to the autonomic nervous system. Since the amino acid, L-tryosine, is a normal component of legume plant protein, a more detailed investigation of the quantitative aspects of this entity in leguminous plant protein has been initiated. Alkaloid fractions of fresh alfalfa and Ladino clover have also given positive bloating responses. The influence of saliva in bloat production is being investigated. Sheep, with their parotid and submaxillary salivary ducts ligated, are being used to determine if sheep with limited salivary secretion, bloat less on alfalfa pasture than untreated sheep. However, thus far no bloat has occurred in either group. A new plastic cannula for gastrointestinal studies has been developed. The cannula appears to be quite adaptable to many physiological studies. Other aspects of this project have involved improving equipment and facilities for a detailed study of the physiology of the eructation mechanism of ruminants.

In 1962, in cooperation with the Agricultural Experiment Station of the University of Maryland, and in informal cooperation with the Animal Husbandry Research Division, ARS, at College Park and Beltsville, Maryland, extracts of alfalfa and Ladino clover were administered to sheep. The bloat response indicated that these legumes contain parasymphathomimetic and sympathomimetic compounds in sufficient quantities to be a major factor in natural bloat. Tyrosine, administered orally to sheep, in amounts which could be consumed from green legumes, proved to be sympathomimetic. However, when tyrosine was administered in cottonseed oil, no bloat resulted. In vitro fermentation studies with C^{14} labelled tyrosine indicated that the tyramine complexes with the lipid fraction of the rumen contents.

These results support those in the literature which indicate that the autonomic nervous system is the major system controlling eructation. Results with alkaloid and other extracts of legumes indicate that the para-symphatholytic activity necessary for inhibition of eructation is present in legumes. The indication that the amine derivatives of tyrosine form lipid complexes offers a possible explanation for the usefulness of oils in controlling bloat.

In 1961, in cooperation with the University of Minnesota, an electromagnetic method was adapted and used for the determination of blood flow to the various compartments of the ruminant stomach and to study factors affecting this flow. A better understanding of the factors important to accurate calibration of the instrument has been developed. Results of flow measurements indicated that a blood flow to the forestomach markedly increases during and following feeding. This increased flow appears to be primarily confined to the forestomach and is associated, at least in part, with absorption from reticulorumen.

In 1961, the Mississippi Agricultural Experiment Station, State College, under a cooperative agreement with the USDA, paddocks of hop clover, alfalfa and lespedeza were planted. However, due to unfavorable climatic conditions, insufficiently pure stands were attained for use in producing bloat, therefore, no bloat data was collected for the above-mentioned legumes. Crimson clover was grazed by selected bloater and non-bloater steers. The steers were grazed for 24 days, which allowed 20 days of grazing while the clover was in a bloat-producing stage, and 4 days of grazing in which the steers were not bloating. Rumen samples were taken twice during the grazing period. The first sample was removed after 9 days of grazing and a second sample was removed on the 24th day of the period, thereby giving a rumen sample during the bloat and non-bloat producing stages of growth. The rumen samples are to be analyzed for short chain volatile fatty acids.

In 1962, at the Mississippi Agricultural Experiment Station, rumen samples collected in the spring of 1961 have been analyzed for volatile fatty acids (VFA's). In this study selected bloater and non-bloater steers grazed clover for 1½ hours morning and afternoon. Rumen samples were taken by stomach tube before and after grazing. When the VFA data (milliequivalents of VFA's per 100 ml. rumen fluid) were subjected to statistical analysis the total VFA concentration for bloaters were significantly higher than those for non-bloaters. Significantly more total VFA's were present in samples

taken at 9:30 (after grazing) than those at 7:30 (before grazing). There was a highly significant difference in VFA's present in the rumen fluid. There were significantly more total VFA's in the 9:30 bloater rumen samples than in the 9:30 non-bloater samples.

In 1961, in cooperation with the Wisconsin Agricultural Experiment Station, Madison, efforts were directed toward identifying and characterizing the enzymatic systems of ruminal digestion of leguminous material thought to play a role in the production of increased viscosity of ruminal contents, since it had been demonstrated previously that this is an important factor in producing the physical character of ruminal contents conducive to bubble retention or froth production.

The qualitative determination of pectin methyl esterase (PME) activity was studied by the change in acidity of a pectin solution and by the formation of a pectic gel in the presence of Ca ions and pectin. Alfalfa extracts and a commercial pectinesterase solution always gave a positive reaction in both qualitative tests during the feeding of alfalfa hay to cows - the (centrifuged) ruminal fluid contained no detectable PME activity. The enzyme activity appeared to be associated with the particulate or solid matter.

As a model for estimating the enzymic increases in the viscosity of stomach contents of cows, factors controlling pectin gel formation were investigated, using a commercial pectinesterase. From these studies, optimal amounts of pectin methyl esterase, NaCl, CaCl₂ and pectin were suggested which should give a firm gel of Ca pectate in 30-40 minutes.

When fresh alfalfa was extracted with NaCl and added to pectin, the solution gelled within 5 minutes; alfalfa hay extract gelled after 25 minutes. The gelled solutions appeared to liquify enzymically. Higher NaCl concentrations seemed to change the rate of gelling and affect the ultimate equilibrium of the pectin containing system.

Extracts from fresh alfalfa treated with strained, undialyzed ruminal fluid, increased the relative viscosity of pectin to a very high value in 30-40 minutes. Using extracts from centrifuged ruminal fluid, the enzymic increase in the viscosity of pectin was much slower. The heated extracts did not increase the relative viscosity of pectin. The enzymic increase in relative viscosity of pectin also paralleled the protein concentration both for centrifuged and strained ruminal extracts of fresh alfalfa.

High concentrations of NaCl in the extracting buffer appeared to increase the amount of pectin methyl esterase extracted or its enzymic activity. The same NaCl concentrations in the enzymic reaction mixture containing pectin, added CaCl₂ and dialyzed fresh alfalfa extracts, caused only a small increase in the relative viscosity of pectin. The higher NaCl concentration is believed to influence the ultimate gel strength and to extend the time required for orientation of pectin molecules to permit cross-bridging in the presence of the optimal CaCl₂ concentration.

A crude bacterial pectin degrading enzyme system was prepared for use in identifying the possible pectic substances in frothy, ruminal contents of pasture-bloating cows. The properties of the culture filtrates were studied. Viscometrically the filtrates initially degraded pectic acid 3 to 8 times more rapidly than they did pectin. Their action was like that of an endo-polygalacturonase. Alkyl aryl sulfonate lowered only the initial enzyme activity of these preparations. Dialyzed and lyophilized preparations were retained for further enzymic studies.

When samples of ruminal froth were incubated with various enzymes and inhibitors, a little froth-breaking was observed only in the presence of pectinase, alkyl aryl sulfonate, alpha amylase, Rhozyme H-39, and cellulase. On repeated tests, a higher concentration of cellulase was the most effective preparation against froth stability.

In 1962, at the Wisconsin Station, the feeding of lush, rapidly-growing alfalfa (top 3-4 inches) following 2-3 days on grass or alfalfa hay, produced experimental bloat in cattle. Although the "Before Feeding" sample contained froth, the "After Feeding" samples of frothy digesta were very stable and the froth remained firm and did not break for several hours. Other properties of the bloating process and characteristics of the expressed foamy fluid were observed.

The type of metabolism of the accessible pectin and other associated polymers in the macerated alfalfa plants rather than a large increase of these substances in the rumen cavity may well determine whether the animal bloats. Initial investigations have begun on pectin and pectic substances as the available substrates along with other substances which surround or may limit their release in the rumen when rapidly growing alfalfa is eaten by the cows. Penetration of the waxy surface layers and the cellulose microfibril of these plants may influence the accessibility of these pectin polymers for complete metabolism to products innocuous to bloating.

In continuing studies on factors which influence pectin gel formation by PME, centrifuged rumen fluid produced a firmer gel than water. On the other hand, the rumen precipitate from the centrifuged strained rumen fluid inhibited the pectin gelling when it was added back to the supernatant (centrifuged fluid). Similarly, the properties of pectin methyl esterase prepared in water differed from those reported previously where the enzyme was prepared in the more protective phosphate buffer-sodium chloride medium. This discrepancy will be further investigated.

For ruminal digestion of starchy feed, the role of starch-digesting enzymes and other related substances in the rumen as possible causes of feed-lot bloat, has been initiated. Two experimental systems have been used to evaluate qualitatively and quantitatively the action of starch-digesting enzymes in a commercial source and in rumen fluid from a cow presently feeding on hay.

One method, the starch solution color test, was only useful with soluble starch as the substrate and in these experiments served as a qualitative measure of enzyme activity. The other technique, the agar plate method, measured the quantitative enzyme activity and thus shows great promise for testing inhibitors of these enzymes. This plate method may be used to distinguish between the two fractions of starch. Cornstarch was used with good success when the starch-digesting enzymes were tested on the agar plate method; but this substrate did fail to respond in the solution test. Other reactions are being explored with the agar plate technique.

In 1962, at the National Animal Disease Laboratory at Ames, Iowa, and in previous cooperation with Cornell University at Ithaca, New York, it has been found that eructated gases enter the trachea at pressures approximating those occurring in the esophagus during the expulsive phase of eructation and penetrate deeply into the lungs. Elevation in systemic arterial CO_2 values occur with eructation of this gas. That this increase in arterial CO_2 is not due to the reflex activation of pulmonary arteriovenous shunts is indicated by the fact, among others, that arterial levels greatly exceed the CO_2 content of simultaneously collected venous blood samples. The maximum increase in arterial CO_2 occurs in conjunction with eructation, that is, the arterial samples collected at the level of the carotid artery would have been in the pulmonary circulation at the time of eructation, indicating that there is direct passage of CO_2 into the pulmonary arterial circulation during eructation. This increase in arterial CO_2 is not due to breath-holding or increased intratracheal pressure alone.

That the pulmonary system provides a route of absorption of eructated gas is further substantiated by the finding that various gases, including CO_2 , CO , H_2S , and O_2 , after being placed in the rumen, are more readily capable of causing changes either in blood-gas levels or in the physiologic activity of the animal when the trachea is patent and capable of receiving these gases during eructation.

The following observations have been made:

- 1) A nasopharyngeal sphincter closes during eructation and prevents expulsion of gas through the nose.
- 2) The nasopharyngeal sphincter is formed by the levator veli palatini and oral pharyngeal constrictor muscles.
- 3) The oral pharyngeal constrictor muscles of the ruminant are involved in the absorption of gas by the blood from the lungs.
- 4) The glottis remained open during the active phase of eructation, and eructated gas was forced into the trachea.

These observations, together with experiments reported elsewhere on the absorption from the lungs of eructated gases, indicate that a significant amount of eructated gas is forced into the lungs.

Two cows, with ruminal and tracheal fistulas, were used in a series of experiments to determine the depth of penetration of eructated gases into the lungs and the effects of these gases on the flavor of milk. They were also used in experiments designed to compare the lungs to the rumen in effectiveness as absorptive-routes for substances causing off-flavors in milk.

Substances causing off-flavors in milk were detectable in the milk much sooner when given by the lung route than when given by the digestive tract route. For example, detectable off-flavors appeared within 15 minutes after substances were introduced into the lungs, whereas, when substances were introduced into the rumen, it required at least 30 minutes.

It was necessary to incubate macerated onions with rumen ingesta before off-flavors were detected by organoleptic methods in the milk when the lung route method of administration was used. Introduction of macerated onions into the rumen gave rise to a strong off-flavor. The off-flavor appeared sooner when the eructated gases were permitted to enter the lungs than when they were blocked from entering the lungs. Eructated gases also were found to play a role in the transmission of ethyl and amyl acetates from the rumen to the milk.

D. Preparedness for Diagnosis for Foreign Animal Diseases.

In 1961, at the Plum Island Animal Disease Laboratory, studies conducted in swine with rinderpest virus, the susceptibility of this species to the virus was established, and swine have been shown to harbor the virus in bone marrow for as long as 36 days post-inoculation. Complement-fixing antibodies were demonstrated in the serum of swine and in cattle, indicating the usefulness of this test for diagnosis of rinderpest in these species. Beta propiolactone has been found useful for inactivating rinderpest virus in immune serum, and it has been shown to have no effect upon virus-neutralizing or complement-fixing antibodies.

In the course of preparation of materials for diagnosis of Teschen disease, limited observations were permissive in animals. With the swine tested, pigs more than 10 weeks of age were resistant to infection by experimental inoculation.

Competence in diagnosis of vesicular exanthema was achieved during the reporting period and methods were developed for complement-fixation and agar-diffusion tests, using vesicular exanthema virus or serum from convalescent animals.

The hemadsorption test for diagnosis of African swine fever has been applied during the reporting period at the Plum Island Animal Disease Laboratory, and the virus has been serially passaged in tissue cultures.

In 1962, at the Plum Island Animal Disease Laboratory, capabilities for diagnosis of fowl plague, contagious bovine pleuropneumonia, rinderpest, foot-and-mouth disease, Teschen disease, African swine fever, and vesicular exanthema have been obtained. During the reporting period, work has been conducted on rinderpest, African swine fever and foot-and-mouth disease in diagnostic investigations. In work with rinderpest virus, it has been shown that the virus may be transmitted from swine to cattle. However, transmission from infected cattle to swine had not been previously shown. In work conducted at this laboratory, a group of 15 pigs became infected when housed with 2 steers showing signs of rinderpest.

The relationship of distemper, measles and rinderpest has been studied. Monkeys injected with rinderpest virus developed rinderpest antibodies, but not distemper or measles antibodies. Monkeys injected with distemper virus developed distemper and rinderpest antibodies, but not measles antibodies. Puppies infected with rinderpest virus developed both rinderpest and measles antibodies, but not distemper antibodies. However, the puppies apparently were protected against challenge with distemper virus; puppies infected with measles virus developed both measles and rinderpest antibodies. Such puppies were also protected against challenge with distemper virus. Each of the three viruses produced homologous, and in some instances, heterologous antibodies in an alien host. From this work, it may be concluded that after natural or artificial exposure to measles or distemper virus, cattle will not develop antibodies to rinderpest virus that would interfere with the interpretation of serologic tests for rinderpest.

Comparative studies were also conducted with a strain of virus diarrhea and rinderpest viruses. The results of this work suggest that there is no essential serologic or immunologic relationship between the viruses of rinderpest and virus diarrhea insofar as facts are known today.

Rinderpest virus has also been examined by thin sectioning and electron-microscopy techniques. In this work, it has been shown that the effects of infection were visible at 3 hours but development of virus bodies and possible precursor structures occurred later. Rinderpest virus apparently develops within or contiguous to mitochondria as evidenced by small particles of 40-60 mu. which may precede the complete virus of 150-300 mu. in diameter. Virus develops slowly in cells and the final morphological form was visible 7-8 days after infection.

E. Toxicology and Pathology Related to Insecticides

In 1961, at the Toxicological Investigations Laboratory at Kerrville, Texas, in cooperation with the Entomology Research Division, intramuscular and subcutaneous injections of phosphorus-32 labeled Bayer 21/199 as saline suspension, oil suspension/solution, or solution indicated that considerable percentages of the injected materials would be present at the injection site for more than 2 months. Encapsulation of the material would further delay absorption and was observed to occur. Muscle and fat biopsies of a calf, given 1.86 mg/kg of carbon-14 labeled phosphamidon (Ciba) were negative for the material. Biopsies were performed 7 days after dosing. Milk samples from dairy cattle, sprayed with 0.25-0.5 percent equivalents of Shell compound 4294 labeled with carbon 14 or phosphorus-32, indicated apparent residues of 5 to 23 parts per billion. These tiny quantities have not been conclusively identified. Dairy cows were sprayed with phosphorus-32 labeled General Chemical Company compound 4072 at 0.25 percent equivalent. One of the cows received an ADP formulation of xylene and lanolin solution of the compound, the other a regular water emulsion. Apparent residues were present in milk but not identified. The apparent material in milk was reduced by 50% by the ADP formulation.

Shell Compound 4294 (alpha-methyl benzyl 3-(dimethoxyphosphinyloxy) crotonate) was found safe for general livestock use on cattle, sheep, goats, and hogs at the proposed concentration of 0.25 to 0.5 percent in sprays. Toxicity appeared at 0.5 percent in goats; 1.0 percent in sheep, and at higher doses in cattle and hogs.

Ruelene (4-tert-butyl-2-chlorophenyl methyl methyl phosphoramidate) was studied extensively, both as an oral and as a dermal treatment. The maximum non-toxic dosage, administered orally, was 25 mg/kg for young calves; 50 mg/kg for cattle; 100 mg/kg for Angora goats, and 150 mg/kg for sheep. The maximum non-toxic concentration in sprays and dips appeared to be 1.0 percent for young calves and Angora goats; 2.0 percent for sheep and cattle. Cattle were not harmed by 16 treatments with 0.5 percent sprays applied at weekly intervals.

Virginia-Carolina Chemical Co. Compound V Cl-13 (O-2,4-dichlorophenyl O,O diethylphosphorothioate). An extensive study of this compound indicated that the maximum safe concentration for use as a spray would be 0.25 percent for calves and goats; 0.5 percent for sheep, and 2.0 percent for cattle, provided the animals are in good flesh. Emaciated sheep were poisoned by 0.5 percent and emaciated Angora goats by 0.25 percent.

Oral toxicity of miscellaneous insecticides. A total of 39 compounds were evaluated for oral toxicity during the year in cattle, sheep, or horses, using only a few animals in each study to give entomologists an indication of the relative toxicity of the compounds prior to their use of them.

Dermal toxicity of miscellaneous insecticides. Four techniques of dermal application were utilized this year in these studies. One method was the customary spraying of animals with 2 to 4 quarts of emulsion or suspension of the compound. A total of 34 compounds were applied in this manner to young dairy calves or cattle.

The second method utilized the same type of emulsions and suspensions, but for particular entomological studies only the hindquarters were treated. Nine compounds were studied in sheep. These materials were promising ones for use in screwworm control.

A third method utilized a procedure now popularly called "pour on." This involves the use of relatively high concentrations with reduced volume simply poured along the dorsal midline of cattle, primarily to obtain cattle grub control by systemic action. A total of 17 compounds were used in these studies.

The fourth method involved the use of high concentrations of insecticide in low volumes of a volatile solvent applied as a mist to the hair. A total of 25 compounds were applied in this manner.

Injectable insecticides. Ten compounds were utilized in these studies by entomologists.

2-pyrimidine aldoxime methiodide (2-PAM) was studied as an antidote for Co-Ral poisoning. It did not perform satisfactorily, atropine giving better results even though not entirely satisfactorily.

SKF 525A was not effective as an antidote, again atropine being superior.

Residues of Insecticides in Animal Tissue and Milk:

Sevin. There were no detectable residues of this compound in cattle, sheep, goats, and hogs sprayed 4 times at 4-day intervals with 1.0 percent suspensions. There were no detectable residues in cattle after feeding 50 and 200 parts per million in the total diet for 4 weeks.

Virginia-Carolina Compound V C1-13. Preliminary studies indicate that this compound is stored in the fat of treated animals. Critical studies are being considered for next year.

Oral Toxicity of Herbicides Administered Daily to Sheep: Eleven herbicides were studied in sheep this year. Sheep survived massive dosages for prolonged periods of compounds of 2,4-D; 2,4,5-T; 2-methyl-4-chlorophenoxyacetic acid, and of 2,2-dichloropropionic acid.

Simazine, Atrazine, and dinitrophenol were the most toxic materials studied. The alkamolamine salts of dinitrophenol were much less toxic than the phenol.

In 1962, at the Kerrville, Texas, laboratory, many of the treatments summarized here were administered by entomologists of the Entomology Research Division as a part of their insect control research. ADP personnel observed these animals for evidence of toxicity. Other studies were entirely the responsibility of ADP.

ENT 17,470 (Virginia-Caroline VC 1 - 13) was not toxic to cattle fed 3 mg/kg of the compound in their feed daily for 10 days.

ENT 19,763 (Neguvon, Dipterex, Bayer L 13/59) was toxic for cattle given a single oral dose of 100 mg/kg orally in capsules, but was not toxic at 50 mg/kg, whether the compound was in its technical form or combined in a polymer.

ENT 20,738 (DDVP, Vapona) Horses were not poisoned by doses of 50 or 100 mg/kg of the compound mixed with their feed a single time.

ENT 20,852 (Butonate). Cattle were not harmed by mixing 5 or 10 mg/kg of Butonate with their feed daily for 10 days. Horses given 25 or 50 mg/kg as single doses with a stomach tube showed diarrhea at 25, depression at 50 mg/kg. Mixing Butonate with feed of horses at 10, 25, or 50 mg/kg did not affect them.

ENT 23,708 (Stauffer 1303-E). Spray applications of 0.1 percent and higher poisoned dairy calves 1 to 2 weeks of age. Cattle were not poisoned by 0.05 or 0.1 percent sprays.

ENT 24,650 (Dimethoate, Cygon, American Cyanamid). Dimethoate was not toxic to cattle given 15 mg/kg orally, either as the technical compound or combined in a polymer.

ENT 24,969 (General Chemicals 4072). G.C. 4072 was not toxic to cattle given 2.5 mg/kg mixed with their feed daily for 10 days nor when applied in 250 ml. quantities of 1 percent solution in oil or suspension in water poured along their backs. A horse was unaffected by a single dose of 50 mg/kg of the compound mixed with feed.

ENT 24,988 (DiBrom, California Spray Chemical Corp.). DiBrom had, in previous years, been shown to be too highly irritant to use as a regular cattle spray. Procedures recommended by the company for mist spraying daily with 0.67 percent DiBrom in sugar water severely injured the eyes of the cattle so treated, the effects beginning after 5 daily applications.

ENT 25,540 (Bayer 29,493). Cattle were not poisoned by daily doses in their feed of 2.5 mg/kg of Bayer 29,493. Single intramuscular injections of 2.5 mg/kg were not toxic to cattle but doses of 5 mg/kg poisoned 3 of 4 cattle so treated.

ENT 25,561 (Grace-Ack 58-26). Cattle were poisoned by single oral doses of 25 mg/kg of ENT 25,561 administered in capsules.

ENT 25,602 (Ruelene). Horses were not poisoned by single doses of 25 or 50 mg/kg of Ruelene mixed with their feed.

ENT 25,644 (American Cyanamid 38,023). Cattle were not poisoned by feeding them 10 mg/kg of 25,644 daily for 10 days, nor by single intramuscular doses of 10, 15 or 20 mg/kg.

Sheep were unharmed by 25,50 or 100 mg/kg intramuscular injections. Sheep were poisoned by oral drenching at 100 mg/kg. A horse was unharmed by a single dose of 50 mg/kg administered with a stomach tube.

Ent 25,702 (Bayer 39,193). Sprays of 0.5 percent concentration were toxic to cattle. Cattle were not poisoned by oral doses of 50 mg/kg administered in capsules.

ENT 25,705 Stauffer R-1504). Cattle were not poisoned by sprays containing 0.5 percent of 25,705 but were poisoned by pouring 250 ml. quantities of 2 percent oil solutions or water suspensions along their backs. Single oral doses of 10 mg/kg were tolerated but 25 mg/kg poisoned cattle. Feeding cattle 2.5 mg/kg daily for 10 days did not produce poisoning.

ENT 25,712 (Bayer 37,289). Cattle given single oral doses in capsules at 10 mg/kg were unaffected.

ENT 25,714 (Bayer 38,333). Sprays of 0.25 percent were toxic to cattle. Single oral doses of 10 mg/kg in capsules poisoned 3 cattle so treated.

ENT 25,725 (Bayer 42,600). Sprays of 0.5 percent and oral doses of 25 mg/kg in capsules poisoned all cattle so treated.

ENT 25,749 (Shell 7394). Dairy calves 1 to 2 weeks of age were not affected by oral doses of 15 mg/kg but were poisoned by doses of 25 and 50 mg/kg.

ENT 25,763 (Hercules 7522H). Dairy calves 1 to 2 weeks of age were poisoned by single oral doses of 10 mg/kg and higher. Cattle were not poisoned by single oral doses of 100 mg/kg administered in capsules.

ENT 25,766 (Dow Zectran). Angora goats were not poisoned by 0.25 percent concentrations of Zectran.

ENT 25,769 (Stauffer N-2310). Cattle were not poisoned by single oral doses of 10 mg/kg administered in capsules.

ENT 25,780 (Hooker 1422). Sprays containing 0.25 and 0.5 percent of 25,780 were not toxic to cattle.

ENT 25,795 (Stauffer N-2599). Dairy calves 1 to 2 weeks of age were poisoned by oral doses of 12 mg/kg but not 6 mg/kg. Yearling cattle were poisoned by single oral doses of 20 mg/kg administered in capsules.

ENT 25,797 (Stauffer N-3047). Dairy calves were poisoned by single oral doses of 15 mg/kg and higher. Doses of 8 mg/kg were tolerated. Yearling cattle were not poisoned by 15 mg/kg.

ENT 25,798 (Stauffer N-3054). Dairy calves were poisoned by oral doses of 8 mg/kg and higher and were killed by such doses of 30 mg/kg. Cattle were poisoned by single oral doses of 10 mg/kg administered in capsules.

ENT 25,799 (Stauffer N-3055). Dairy calves were poisoned by single oral doses of 10 mg/kg and higher and were killed by doses of 20 mg/kg and higher. Yearling cattle were poisoned by single oral doses of 10 mg/kg administered in capsules.

ENT 25,801 (Stauffer R-3422). Dairy calves were not poisoned by oral doses of 15 mg/kg but were poisoned at 25 mg/kg. Yearling cattle were poisoned by single oral doses of 20 mg/kg administered in capsules.

ENT 25,810 (Hercules 9699). Dairy calves were not poisoned by single oral doses of 8 mg/kg but were poisoned by doses of 15 mg/kg and higher. Yearling cattle were not poisoned by single oral doses of 13.4 mg/kg administered in capsules.

ENT 26,613 (Rhodia 9895). Dairy calves were not affected by sprays containing as much as 3 percent of this compound. Yearling cattle were unaffected by 2 percent sprays or single oral doses of 100 mg/kg. Feeding 15 mg/kg daily to cattle for 10 days produced poisoning.

Compounds used to induce sexual sterility in insects appear to have a definite cumulative effect upon sheep. Poisoned animals react rather suddenly, even in long-term studies. It is worthy of note that of the two compounds studied, aphoxide, reported to be the more effective chemosterilant, is also by far the more toxic. Such compounds will need to be used most carefully to avoid damage to desirable forms of life.

ENT 26,316 (Apholate). Sheep were killed by single intramuscular injections of 5 mg/kg and by 11 weekly injections, each 0.5 mg/kg. Sheep were killed by single oral doses of 50 mg/kg and by 11 doses of 25 mg/kg administered at weekly intervals. Weekly doses of 5 or 12.5 mg/kg were not toxic after 20 doses, at which time the test was terminated. When administered daily to sheep, deaths followed 11 daily doses of 20 mg/kg; 19 and 22 daily doses of 10 mg/kg; 19 and 25 daily doses of 5 mg/kg and 101 and 128 daily doses of 2 mg/kg. Sheep receiving 1 mg/kg daily have survived 168 daily doses and will be continued in test.

ENT 24,915 (Aphoxide). This compound caused deaths in sheep after 7 and 9 daily doses of 5 mg/kg and after 6 daily doses of 2 mg/kg.

Antidotes for poisoning. Atropine was used extensively throughout the year to save most of the animals poisoned in our own studies and in many of those conducted by the Entomology Research Division. Atropine is still by far the most satisfactory antidote for organic phosphorus and carbamate compound induced poisoning. BAL (dimercaptopropanol) was not useful as an antidote for poisoning by organophosphorus compounds.

Residues of insecticides in meat and milk. These studies were conducted cooperatively with the Entomology Research Division and the companies interested in the compounds.

ENT 17,470 (Virginia Carolina VC 1-13). This study involved sheep, goats and cattle sprayed a single time with 0.5 percent suspensions. Substantial residues appeared in the fats and in smaller amounts in other tissues. The residues declined, reaching extinction at 6 to 8 weeks after treatment.

ENT 19,507 (Diazinon). This study involves only cattle that are sprayed weekly with 0.05 and 1.1 percent suspensions of diazinon. A total of 16 applications are to be made, the final one coinciding with the end of this reporting year. Omental fat samples have been taken and are being analyzed. The test will be reported in full in FY 1963.

Herbicides:

Banvel D (Velsicol). Sheep were not poisoned by doses as high as 1000 mg/kg.

Bandane (Velsicol). Sheep were not poisoned by 200 mg/kg of Bandane but a sheep given 1000 mg/kg was killed. Signs of poisoning were mostly those of central nervous system origin.

Studies were initiated in the area of fungicides that might be used in pasture and field treatment. These were started June 4, 1962.

Captan was lethal for a sheep given a single oral dose of 500 mg/kg. One sheep given 100 mg/kg daily showed signs of poisoning after 3 doses. There is some indication that Captan may have an anticoagulant effect upon the blood.

A Jersey cow was sprayed with carbon-14 labeled Ciodrin (Shell Compound 4294) to obtain data on the excretion of the compound in milk. The conditions imposed by the milk study required the cow to be stanchioned most of the time, giving us an opportunity to study residues of the compound on the hair, unaffected by the normal licking and tail-switching activities of the cow. Under these conditions the residue of Ciodrin on the hair did not decline appreciably in 2 weeks, but the distribution between tight and shedding hairs changed from a proportion of 40/60 to 10/85 in one week, indicating that licking and tail switching would have accounted for removal of more than 80 percent of the applied material had they been permitted. Ciodrin did not appear to migrate along the hair after the spray had dried.

F. Biochemical Effects of Agricultural Chemicals

In 1961 at the Kerrville, Texas, Station, studies were made on sheep penned, then pastured, of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), malic dehydrogenase (MOH), and aldolase in blood sera. Approximately a two-fold increase of serum malic dehydrogenase occurred in sheep after 3 weeks on pasture. No other differences were noted. During the study several individuals showed sudden increases in activity of GOT and aldolase, without clinical signs. Biopsies of liver revealed infiltration with polymorphonuclear leucocytes and moderate infiltration with fibroblasts. There were no remarkable changes in prothrombin time, chlorides, calcium phosphorus, creatinine, non-protein nitrogen, urea nitrogen or uric acid in bloods of sheep receiving representative herbicides for prolonged periods of time.

In 1962 at the Kerrville Station, in a cooperative study with researchers of the Wm. Cooper and Nephews Company, Angora goats and crossbred sheep were given Coopex (phenothiazine plus coroxon) orally, then sprayed immediately or two weeks later with organic phosphorus compounds. A marked potentiation of toxic effects and cholinesterase inhibition was noted in the goats sprayed immediately after drenching when the sprays contained normal concentrations of Delnav or VC 1-13. These effects were not so marked when the sprays contained malathion or Bayer 21/199. The potentiation did not appear when the spraying was delayed two weeks. Sheep drenched, then sprayed on the same day, as were the goats, did not respond to a greater degree than when Coopex was given alone.

The rather remarkable potentiation of effects in goats as opposed to those in sheep indicate that, in the goat, administration of phenothiazine must interfere with the detoxification or metabolism of organic phosphorus compounds present in the body at the same time. There were 3,641 determinations made of the cholinesterase activities in as many samples of blood from experimental animals. There were 119 chemical determinations of hemoglobin and 234 of serum bilirubin. In addition, 119 determinations were made of prothrombin time, 110 of packed cell volume in blood. The results of these determinations, for the sake of understanding and brevity, are reported with the main studies. In addition, as a part of the Kerrville Laboratories safety program, 51 determinations were made of erythrocyte and a like number of plasma cholinesterase activities in the blood samples of the Station's personnel.

In cooperation with the Stephen F. Austin State College at Nacogdoches, Texas, various types of equipment have been investigated to determine their possible use in constructing and calibrating an ultrasonic particle size spectrometer. Techniques for calibrating such a spectrometer have been worked out. The major effort has been directed toward a theoretical and experimental investigation of the scattering of plane acoustic waves by rigid spheres. It has been found that a measurement of the scattering pattern leads to a determination of the size of the sphere. Immediate plans are to continue this investigation using an array of solid spheres as well as liquid drops.

G. Detoxication Mechanism in Cattle and Sheep.

In 1962, at Kerrville, Texas, efforts were concentrated toward successfully developing an analytical method, with appropriate "clean-up" procedures, successfully developing paper chromatographic and electrophoretic methods for identification of 2,4-D, proving both the above methods by use of carbon-14 labeled 2,4-D, and studying the absorption, excretion and tissue residues of 2,4-D in a sheep given a single oral dose of 4 mg/kg.

In the sheep given the 4 mg/kg dosage of 2,4-D maximum concentrations of the compound in blood were reached in 2 hours. At 8½ hours after dosing 50% of the administered dose had been recovered in the urine and 90% had been recovered 28 hours after dosing. In the urine 2,4-D was the only radioactive material found. The sheep was destroyed 4 days after treatment. Tissues obtained at necropsy were analyzed, yielding an average apparent residue of 2.6 parts per billion.

The highest tissue residue occurred in the thyroid gland, amounting to only 0.56 part per million, but this was far more than occurred in any of the other tissues. This finding lead us to wonder whether or not the 2,4-D had been mistaken in the body for a precursor of thyroxine such as mono- or diiodo-tyrosine.

H. Cytological Responses to Antiparasitic and Other Agricultural Chemicals.

In 1962, at Kerrville, Texas, it was found that Apholate (ENT 26,316) administered to sheep in small daily doses regularly produced marked decreases of the white cells, particularly the lymphocytes and the polymorphonuclear leukocytes. Marked decreases in blood platelet numbers were noted. The red blood cells, in number, volume and hemoglobin content were not significantly affected. Serum bilirubin and serum prothrombin time were not appreciably affected. Necropsy findings usually involved the circulatory system. All dosages in excess of 1 mg/kg daily have killed the sheep so treated.

Histological examinations, gross and microscopic, of the tissues of sheep poisoned by several organic herbicides, show that the primary effect of those materials is upon the kidney, followed closely by the lungs. Other tissues may be involved with hemorrhages and other types of generalized changes.

During this reporting period 87 sets of tissues were obtained for processing. A total of 3,648 routine hematoxylin-eosin stained sections were prepared. Special staining was done for 310 slides, including periodic acid - Schiff, Gomori, Gridley fungus, Giemsa, Gram's, and amyloid and fat stains. Wright's stain was used for 140 blood smears.

I. Poisoning by Plants.

In 1961, at the Logan, Utah, poisonous plant field station, studies were made to determine the cause of congenital cyclopia-type malformation in lambs for the sixth consecutive year in cooperation with the U. S. Plant, Soil and Nutrition Laboratory of the Soil and Water Conservation Division, and the Utah Agric. Exper. Station. Veratrum californicum, commonly called False hellebore, Wild Corn, and Skunk cabbage, fed to 51 bred ewes for 30 days, caused a congenital cyclopia-type malformation in the lambs of 17% of the ewes, and fetal deaths in 29 percent. This is the first time a poisonous plant has ever been experimentally proven to be associated with a congenital deformity in animals. The Veratrum californicum plants, collected at different locations, showed a marked variation in their ability to cause toxic symptoms in ewes and congenital deformities in lambs. The commercial alkaloids of Veratrum californicum caused toxic symptoms similar to the veratrum plants when fed to sheep. Veratrum californicum is definitely a poisonous plant for sheep, causing characteristic toxic symptoms and pathological changes in certain organs. A congenital cyclopia-type malformation has been occurring for the past 60 to 70 years in lambs born from ewes bred in August and early September on certain areas of the summer ranges in the Boise, Sawtooth, and Challis National forests. This deformity has always been looked upon by the livestock men, and many individuals in scientific research, as a hereditary disease. The number of affected lambs has ranged from 1 to 25 percent in individual herds of 3,000 to 10,000 ewes each. The congenital anomaly is related to a condition of prolonged gestation in ewes bearing the affected lambs. Such ewes are sold as culls or die during parturition and results in a two-fold loss which is estimated to range from \$15,000 to \$100,000 annually for the sheepmen in southeastern Idaho.

The investigations of locoweed poisoning in sheep were indicated in the previous work reported in ADP a7-7 annual report 1960, to cause abortion and congenital deformities in sheep. Loco poisoning in ewes has often been suspected by the sheepmen as a possible cause of increased number of dry ewes and congenital deformities in lambs when poisoning occurs during the gestation period. In 1960, 9 of 22 ewes aborted. One aborted fetus and 3 full-term lambs had from slightly - to - marked twisted front legs. In further investigation of this problem, twin full-term lambs were born from a ewe fed Oxytropis lambertii (locoweed), with congenital deformities of front legs. One lamb showed contracted pastern joints of both front legs, and the other twin had contraction of the pastern joint in only the right front leg. Both animals walked on the distal end of the metacarpus bone. Three rams fed locoweed daily showed marked decrease in physical vigor and decrease in semen quality, which was reflected in the morphology of the sperm collected by electrical ejaculation.

Fourteen ewes were maintained on a locoweed-free diet after suffering from loco poisoning and abortion. The ewes were re-bred the following year. All conceived at the first breeding period and gave birth to normal full-term lambs. This indicated that there is little or no danger of a carry-over of reproductive disorders following the recovery of locoweed poisoning in ewes. One 2-year old Holstein cow was fed locoweed (Astragalus lentiginosus) from

the 41st to her 100th day gestation. She gave birth to a full-term normal calf, as compared to 2 heifers fed the previous year and aborted fetuses with twisted front legs.

Studies to determine the etiology of a congenital deformity in calves, commonly called "crooked calf disease," have been continued for the 6th consecutive year in cooperation with the U. S. Plant, Soil and Nutrition Laboratory of the Soil and Water Conservation Division. The disease has been found to occur in Idaho, Washington, Montana, Wyoming, Oregon, California, Nevada, and Utah. It is likely that the disease is occurring in many other States, but not recognized because the livestock men have always considered the anomaly to be of hereditary origin and kill the affected calves soon after birth. The disease has caused an estimated loss of \$500,000 to \$1,000,000 annually to the livestock industry.

The disease has been known to occur ever since records were kept on range cattle. The Animal Disease and Parasite Research Division researchers were the first to start a detailed scientific study to determine the etiology of this deformity. A congenital deformed calf, typical "crooked calf disease," was reproduced for the second time by feeding a Holstein heifer Lupinus sericeus and lead daily from the 56th to the 115th days of the gestation period, but 9 heifers, fed lupine without lead, all gave birth to normal calves. Lupinus argenteus, fed to 5 ewes twice daily for 45 days starting on day of breeding, caused marked general body weakness and slight liver dysfunction. All ewes conceived on first breeding, and gave birth to full-term live normal lambs.

The halogeton plant (Halogeton glomeratus) has spread from its original site, first identified in the United States in 1934 at Wells, Nevada, to all eleven western States. The plant is very difficult to eradicate once it becomes established due to its prolific ability to produce large numbers of resistant and dormant seeds.

Investigations from this Station have shown that sheep losses from halogeton poisoning can be prevented by supplementing the feed with dicalcium phosphate. The supplement has been effective when added to grain or alfalfa pellets at the 5% level and fed 1/4 lb. daily when animals are grazing in the halogeton-infested areas.

The use of dicalcium phosphate as a feed supplement has saved the sheep industry many thousands of dollars by preventing losses from halogeton poisoning. Dicalcium phosphate is the only mineral supplement which will provide 100 percent protection. Bone meal, a mineral supplement recommended for livestock for many years, makes the animals more susceptible to halogeton poisoning.

In 1962 at Logan, Utah, the study on the cyclopia-type malformation in lambs, which has been shown to be caused by the maternal ingestion of Veratrum californicum, has been continued in cooperation with the USDA Soil, Plant and Nutrition Laboratory, Ithaca, New York. After it was discovered that

Veratrum californicum was a poisonous range plant and when ingested by ewes early in the gestation period would cause congenital deformities in the lambs, the sheepmen who kept their animals away from the plant during the breeding season have eliminated this disease condition. These men reported that it was the first time since they had been in the sheep business that they did not suffer losses from this deformity. It is estimated that the men who complied with the recommendations to keep their sheep away from the Veratrum californicum plant during the breeding season saved from \$1,000 to \$5,000 each in their 1962 lamb crop.

Various etiological agents are known to cause congenital malformations. So as to eliminate the unknown variables of high altitude (anoxia), transportation and the drying of plants, the main experiment was conducted on the range in Muldoon Canyon, Idaho. The experiment was specifically designed to associate the incidence of congenital deformities, severity of deformities, and the specific time of insult on the developing embryo with this disease condition.

Based on this year's work additional and useful information regarding this disease was obtained. Malformed lambs were born to ewes that were fed Veratrum californicum for 15, 20 and 30 days after the day of breeding. No malformed lambs were born from ewes fed the plant 5 and 10 days from the day of breeding. The incidence and severity of the malformations could not be associated with the degree of clinical toxic symptoms of this disease in the ewe. Embryonic and fetal deaths appear to be associated with this disease process; however, more controlled data and information is needed regarding this one aspect of the disease. The commercial veratrum alkaloid did not induce any malformed lambs, but did produce typical symptoms in the ewes.

Some of the experimental plant (Veratrum californicum) was harvested and hauled to Logan, Utah. After being dried and chopped, it was stored for 3 months and fed to ewes. Malformed lambs were produced under such procedures, but the incidence of malformations was lower than with the green (undried) plant. More information is needed regarding the degree of toxicity associated with the various stages of maturity of the plant, various locations where grown, and proper method of storage to maintain toxicity.

The substance in the plant responsible for causing the deformity is unknown. Further studies have been designed to make this determination. Early investigations have shown that the veratrum plant contains various alkaloids. A chemical analysis was made of Veratrum californicum by Physics and Chemical Investigations of the National Animal Disease Laboratory and showed the specific species to contain alkaloids.

Commercial veratrum alkaloids of cevadine, V. alpha and V. sulfate were obtained to determine their effect on foetus of ewes and chick embryos. Cevadine fed to ewes at 5 mg. per kilogram of body weight caused clinical symptoms identical to poisoning by Veratrum californicum, but failed to cause congenital malformations in lambs when fed at 3 mgs. per kilogram of body weight for the first 15 days of gestation. Cevadine, V. alpha and V. sulfate caused marked retardation in growth and development of chick embryos. Inoculations were made at various levels into the egg yolk from 24 hours before start of incubation to 7 days after incubation.

It is difficult to evaluate, at this time, the relationship of lupine or lupine plus lead to the etiology of "crooked calf disease" until more experimental data is available. As epidemiology studies are made and experimental feeding trials carried out, they seem to indicate, more each year, that the causative agent for "crooked calf disease" is of plant origin.

Clawson and Huffman, in 1937, showed that the ingestion of Tetradymia glabrata by range sheep would cause "Bighead disease." The results in experimental feeding trials have been very erratic. The plant was poisonous and in toxic doses would commonly cause death with severe liver and kidney damage. The swelling of the head would not always be produced even though the animals were in direct sunshine. This problem has always been of much concern to us in the study of the effects of poisonous plants on livestock.

There may be another plant the sheep must eat, either before or along with Tetradymia glabrata, in order to make the animal sensitive to the sun in order to cause marked edema and swelling of the head. The purpose of this experiment was to make a preliminary study to determine if further investigations would be justified another year.

The "Bighead" disease was unable to be reproduced by feeding Tetradymia plus alfalfa hay, Tetradymia plus green grass, Tetradymia alone, or Tetradymia plus big sage. Two animals died with severe liver and kidney damage, and all others were sick and off feed for three days following the feeding trial.

Investigations to determine the relationship of locoweed poisoning to congenital deformities in cattle and sheep have been continued. Previous studies reported in annual report of ADP a7-7, 1960 and '61, showed congenital malformation in legs of lambs produced by maternal feeding of Oxytropis lamberti, Astragalus pubentissimus and A. lentiginosus.

In the years when there is considerable rainfall on the winter ranges during September, October, and November, with moderate temperatures and no snow, loco plants grow very profusely. As the animals come from the summer ranges to the winter ranges in late October and early November, the locoweed have all made new growth and are from 4 to 10 inches in height. Once the animals start eating the plants they tend to ignore other range forage and look only for the locoweeds. In the years when such conditions occur, they are always followed by serious livestock losses from loco poisoning. Bands of sheep suffering from loco poisoning have been known to have as much as 80 percent abortions with a 15 percent death loss in the ewes.

There is no known prevention or treatment for the disease at this time. The plants are so widespread that it is impractical to attempt control of the plants by herbicides. Further studies are necessary to determine the effect of possible alleviators to prevent the poisoning in the animals and new methods for eradicating the plants.

In 1962, under a PL 480 grant to the Instituto Biologico, Sao Paulo, Brazil, Mascagnia pubiflora was found to be abundant, the leaves were toxic, and the plant was causing extensive losses in cattle. The lethal dose was found to be about 4 percent of the body weight. The symptoms and lesions were determined and guinea pigs and rabbits were also found susceptible to its toxicity. Studies are also under way on the isolation and chemical identification of toxic principles of Palicourea marogravii, and Sessea brasiliensis.

J. Toxicity of Herbicides and Herbicide-Treated Plants for Domestic Animals.

In 1962, at Logan, Utah, in cooperation with the Crops Research Division of ARS, and the Utah Agricultural Experiment Station, the following report is submitted.

Since Veratrum californicum has been found to be a poisonous plant for sheep and will cause a congenital cyclopia-type malformation in lambs, marked interest has been stimulated in the sheepmen to know how herbicides may affect its toxicity for sheep. Four 1/100 acre plots of Veratrum californicum plants were laid out in Logan Canyon. One was sprayed at a rate of 2 quarts per acre of 2-4D and one 4 quarts per acre. Two other plots were sprayed with 2-4-5T. One plot was at a rate of 3/4 lb. per acre and the other 1.5 lbs. 2-4-5T per acre. The plants varied from 1 to 2 feet in height, all leaves were dark green color and plants were making rapid growth at the time they were sprayed. Seven days later the leaves of the treated plants were starting to show a rust-brown coloration and growth rate seemed to be retarded compared to the plants on the outside of the plots.

One and three-fourths lb. of the untreated green veratrum plant was required to produce mild toxic symptoms in sheep. Three weeks after the plants were sprayed with 2-4D, 2 1/2 lbs. of the plant failed to cause symptoms while 2 1/2 lbs. of the plants sprayed with 2-4-5T caused mild toxic symptoms.

A herbicide that will kill the veratrum plants without destroying other types of forage or reduce its toxicity to prevent poisoning and congenital malformations in lambs is urgently needed by the sheepmen. Such a herbicide would save time, extensive work and many lambs and ewes that are now being lost from ingestion of the plant.

From this preliminary investigation, 2-4D applied as a spray at a rate as low as 2 quarts per acre was effective in reducing the toxicity of the veratrum plant to sheep, while 2-4-5T seemed to have very little effect at the highest rate of application (1.5 lbs. per acre).

This past year some of the sheepmen have preceded their sheep to the summer range areas and cut the veratrum plants down by hand. If 2-4D can be used as effective on the ranges as it was on the experimental plots to kill the veratrum and prevent toxicity it will save considerable time, expense and labor.

K. Alleviators and Diagnostic Tests for Plant Poisoning.

In 1961, at the Logan, Utah Field Station, dicalcium phosphate was shown to be very effective in the prevention of halogeton poisoning in sheep.

In 1962, in further studies with dicalcium phosphate in relation to halogeton poisoning, it has also been found very effective in the prevention of "dirt eating" in lambs and cattle and stiffness in range lambs at 2 to 4 months of age.

The sheepmen who have followed the recommendations of supplementing their animals free choice the year around with a mixture of 50 lbs. ground salt and 50 lbs. of 18% dicalcium phosphate have suffered only very slight losses from halogeton poisoning and no losses in lambs from "dirt eating" or stiffness of lambs on the range. In determining the effectiveness of dicalcium phosphate as an alleviator to cattle and sheep in the prevention of halogeton poisoning, "dirt eating" in lambs and stiffness in range lambs has saved the livestockmen many thousands of dollars. The saving comes not only in the prevention of animal losses, but in the cost of the mineral. Commercial minerals are sold from \$6.00 to \$20.00 per hundred while salt and dicalcium phosphate mixture may be obtained from \$5.00 to \$6.00 per hundred with uncomparable results on the animals.

Blood tests of sheep fed halogeton show an increase in the potassium and sodium ions and urea nitrogen. Animals fed halogeton plus dicalcium phosphate showed no marked increases of potassium, sodium and urea nitrogen. The calcium and phosphorus ions in dicalcium phosphate seem to be more readily metabolized than from other mineral supplements and their alleviating effect may be found effective in many metabolic disturbances.

L. The Susceptibility of Wild Animals to Foot-and-Mouth Disease.

In 1961, at the Plum Island Animal Disease Laboratory, the susceptibility of horses, cats, rats, dogs, monkeys, frogs, and steers to foot-and-mouth disease virus (FMDV) and its ribonucleic acid (RNA) were compared. They were injected intramuscularly or on the tongue with tissue-cultured FMDV, type A, strain 119, using a dosage proportionate to the weights of the animals. Blood was collected 24 hours after injection and tested for demonstrable virus in steers, mice and tissue cultures. Another group of animals of the same species was treated similarly, using RNA derived from FMDV as the inoculum. Virus was demonstrated in the blood of cats, rats, and steers 24 hours after inoculation with intact FMDV, and after inoculation with RNA. Monkeys and frogs developed a viremia only after inoculation with intact virus. Horses and dogs failed to develop a viremia following inoculation with intact virus or its RNA. Comparable results were obtained in the three test media used, i.e., steers, mice and tissue cultures. A few muskrats trapped on Plum Island were inoculated in the metatarsal pads with FMDV, guinea pig strains A-GB or C-GC. Blood was collected from the animals 24, 48, and 72 hours after inoculation and tested in guinea pigs. The ID_{50/ml}

titers of FMDV in the blood of the muskrats ranged from 3.0 to 3.5 at 24 hours, 2.5 to 4.5 at 48 hours, and 2.8 to 3.8 at 72 hours after inoculation. The titers obtained and the duration of the viremia indicated that the virus multiplied in the muskrats.

In 1962, workers at Plum Island report that previous experiments indicated that monkeys, cats, rats, muskrats and frogs develop viremia after inoculation with FMDV. Of these animals, only muskrats had clinically recognizable lesions. Horses and dogs did not develop viremia after inoculation. When RNA of FMDV was used, only cats and rats had viremia; monkeys, horses, dogs and frogs did not.

Nine chinchillas (chinchilla langiera) were infected by intradermal inoculation of both metatarsal pads with 0.1 ml of 10% suspension of FMDV A-119 representing the 18th guinea pig passage. Within 30 hours, vesicles developed in all inoculated sites. Secondary lesions developed from the 2nd to 9th day postinoculation, and 5 chinchillas died during this time. Blood samples for serologic examination were taken from all animals immediately before inoculation and from surviving animals 14 and 22 days postinoculation. Virus-neutralization tests in suckling mice were conducted using FMDV A-119 and FMDV O-39 as antigens. Neutralizing antibodies against A-119, but not O-39, were present in the sera of the 4 surviving chinchillas 14 and 22 days postinoculation.

M. Environmental Stress as a Contributory Factor in Animal Diseases.

In 1962, at Pulawy, Poland, under a PL 480 grant equivalent to \$76,187, (E21-ADP-7), basic physiological determinations of the normal values of the basic elements in the blood and tissues of poultry have been determined. Preliminary work on the stress influence of adrenocorticotrophin, Pasteurella multocida, and transportation have also been completed. These preliminaries were necessary before engaging in the experiment proper.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Barber, T. L., W. M. Moulton, and S. S. Stone. 1960. The Identification and Typing of Vesicular Exanthema by Complement Fixation and Agar Diffusion Tests. 63rd Ann. Meet. USLSA.

Bezkorovainy, A. 1962. Binding of Thyroxine by Albumin and Acid Glycoproteins of Bovine Plasma. Fed. Proc. 21:407.

Binns, Wayne, and L. F. James. 1960. Halogeton and other oxalic acid poisonings. Proc. Amer. Coll. of Vet. Toxicology, Denver, Colorado.

Binns, Wayne, and L. F. James. 1961. A Congenital Deformity in Calves, similar to 'crooked calf disease,' experimentally produced by feeding lupine and lead. Proc. Amer. Soc. of Animal Production, Western Sect., 12. Moscow, Idaho.

Binns, Wayne, Lynn James, and James L. Shupe. 1961. Psathyrotes annua, poisonous plant for sheep. Vet. Med., 57(6):509-511.

Binns, Wayne, Lynn F. James, James L. Shupe, and K. C. Beeson. 1962. Crooked calf disease produced experimentally. Utah Farm and Home Science, 23(2):35-37.

Boda, J. M., and A. T. Johns. 1962. In vitro fermentation of fibrous and soluble constituents of legumes in the rumen of the cow. Nature 193: 195-196.

Buck, William B., Lynn F. James, and Wayne Binns. 1961. Changes in serum transaminase activities associated with plant and mineral toxicity in sheep and cattle. The Cornell Vet., LI(4):568-585.

Buck, W. B., Wayne Binns, L. F. James, and M. C. Williams. 1961. Results of feeding herbicide-treated plants to calves and sheep. Jour. Amer. Vet. Med. Assn., 138(6):320-323.

Claborn, H. V., R. D. Radeleff, and R. C. Bushland. 1960. Pesticide Residues in Meat and Milk. A Research Report. ARS-33-63.

Claborn, R. V., R. C. Bushland, H. D. Mann, M. C. Ivey, and R. D. Radeleff. 1960. Meat and Milk Residues from Livestock Sprays. Agri. and Feed Chem., 8:6:439-442.

DeLay, P. D., and H. Rozemeyer. 1961. Foot-and-Mouth Disease Virus - Its Behavior in Cattle after Serial Passages in Chicken Embryos and Chicks. Amer. J. Vet. Res., 22:533.

Dougherty, R. W., W. E. Stewart, M. M. Nold, I. L. Lindahl, C. H. Mullenax, and B. F. Leek. 1962. Pulmonary Absorption of Eructated Gas in Ruminants. Amer. J. Vet. Res., 23:205-212.

Dougherty, R. W., K. J. Hill, F. L. Campeti, R. C. McClure and R. E. Habel. 1962. Studies of Pharyngeal and Laryngeal Activity during Eructation in Ruminants. Amer. J. Vet. Res., 23:213-219.

Dougherty, R. W., W. F. Shipe, G. V. Gudnason, R. A. Ledford, R. D. Peterson and R. Scarpellino. 1962. Physiological Mechanisms involved in Transmitting Flavors and Odors to Milk. I. Contribution of Eructated Gases to Milk Flavor. J. Dairy Science, 45:472-476.

Gupta, J., and R. E. Nichols. 1962. A Possible Enzymatic Cause of Viscid Ruminant Contents - Its Relationship to Legume Bloat. Amer. J. Vet. Res., 23:128-133.

Gupta, J., M. H. Crump and R. E. Nichols. 1962. Acid: Base Release of CO₂ from the Bicarbonates of Ruminant Fluid as a Contributing Factor in Bloat. Amer. J. Vet. Res., 23:201-204.

Ivey, M. C., H. D. Mann, R. D. Radeleff, and G. T. Woodard. 1961. Aldrin and Dieldrin Content of Body Tissues of Livestock Receiving Aldrin in Their Diet. J. Agric. & Food Chem., 9(5):374.

Jackson, J. B., R. O. Drummond, W. B. Buck, and L. M. Hunt. 1960. Toxicity of Organic Phosphorus Insecticides to Horses. J. Econ. Ent., 53:4:602-604.

James, L. F., and Wayne Binns. 1961. The use of mineral supplements to prevent halogeton poisoning in sheep. Proc. Amer. Soc. of Animal Production, Western Sect., 12, Moscow, Idaho.

Komarek, R. J. 1962. Mechanistic and Etiological Investigations Concerning Legume Bloat in Ruminants. Ph.D. Thesis, Univ. of Md.

Leffel, E. C., and R. J. Komarek. 1961. Experimental Production of Bloat with Various Chemicals. J. Dairy Science, 44:1771.

Leffel, C., and R. J. Komarek. 1961. The Experimental Production of Bloat and Evidence Concerning Its Natural Cause. Science.

Leffel, C., and R. J. Komarek. 1961. Experimental Bloat and Dietary Factors Affecting Rumen Acid Production. Proc. Univ. of Md., Nutr. Conf. for Feed. Mfrs.

Mendel, V. E., and J. M. Boda. 1961. Physiological studies of the rumen with emphasis on the animal factors associated with bloat. J. Dairy Science, 44:1881-1898.

Moulton, W. M., and S. S. Stone. 1961. A Procedure for Detecting Complement-Fixing Antibody to Rinderpest Virus in Heat-inactivated Bovine Serum. Res. in Vet. Sci., 2:161.

Radeleff, R. D. and H. V. Claborn. 1960. Excretion of Co-Ral in Milk of Dairy Cattle. Agri. and Feed Chem., 8:6:437-439.

Radeleff, R. D., L. M. Hunt, and C. P. Weidenbach. 1961. Toxicity Studies of General Chemical 4072 and Two Related Compounds to Cattle. J. Econ. Ent., 54(5):1051-1052.

Radeleff, R. D. 1962. The Toxicity of Pesticides for Livestock. J. Amer. Oil Chem., 39(3):9-10,12.

Radeleff, R. D. 1962. Absorption and Toxicology of Insecticides. S. Dak. J. of Med. & Pharm. XV(5):200-204.

Radeleff, R. D. 1962. The Role of the Diagnostic Laboratory in Establishing Diagnoses of Insecticide Poisoning. Proc. USLSA.

Roberts, R. H., R. D. Radeleff, and H. C. Wheeler. 1960. Malathion Residues in the Tissues of Sheep, Goats, and Hogs. J. of Econ. Ent., 53:5:972-973.

Roberts, R. H., J. B. Jackson, W. E. Westlake, A. J. Ackerman, and H. V. Claborn. 1960. Residue Studies of Livestock Sprays Containing Sevin. Jour. of Econ. Ent., 53:2:326-327.

Roberts, R. H., R.D. Radeleff, and H. V. Claborn. 1961. Residues in the Milk of Dairy Cows Sprayed with P³²-Labeled General Chemical 4072. J. of Econ. Ent., 54(5):1053-1054.

Rosen, W. G., Hester Fassel, and R. E. Nichols. 1961. The Etiology of Legume Bloat - Nonvolatile Acids. Amer. J. Vet. Res., 22:117-122.

Sellers, A. F., C. E. Stevens, and R. H. Dunlap. 1961. Factors Influencing Gastric Blood Flow in Normal Cattle. Federation Proc. Absts. March.

Shipe, W. F., R. A. Ledford, R. D. Peterson, R. A. Scanlan, H. F. Geerken, R. W. Dougherty, and M. E. Morgan. 1962. Physiological Mechanisms Involved in Transmitting Flavors and Odors to Milk. II. Transmission of Some Flavor Components of Silage. J. Dairy Sci., 45:477-480.

Stone, S. S. 1960. Multiple Components of Rinderpest Virus as Determined by the Precipitin Reaction in Agar Gel. Virology 2:638.

Stone, S. S., and W. M. Moulton. 1961. A Rapid Serological Test for Rinderpest. Amer. J. Vet. Res., 22:18.

Weidenbach, C. P., R. D. Radeleff, and W. B. Buck. 1962. Toxicologic Studies of Ruelene. J. Amer. Vet. Med. Assn. 140(5):460-463.

Williams, M., Wayne Binns, and Lynn F. James. 1962. Occurrence and toxicology of selenium in halogeton and associated species. Jour. of Range Management 15(1):17-22.

Younger, R. L., R. D. Radeleff, and C. P. Weidenbach. 1962. Toxicological Studies of Compound VC 1-13 in Livestock. J. Econ. Ent., 55(2):249-252.

TREATMENT FOR REMOVAL OR CONTROL
OF PARASITES OF DOMESTIC ANIMALS
Animal Disease and Parasite Research Div., ARS

Problem. Parasites of food animals are responsible for losses to livestock producers approximating a billion dollars annually. This estimate, moreover, is conservative since it does not take into account costs of treatment and other control measures. Chemical antiparasitic agents are the most powerful weapons presently available against parasites and the diseases they cause, yet, specific treatments generally have a comparatively short period of usefulness. Many of the currently preferred treatments were unknown a decade or so ago and, in all probability few, if any of those in use today will be primary choices a decade or so hence. Moreover, the growing concern with respect to residues in edible tissues and organs of treated animals and birds necessitates development of control measures other than treatment. The problem is to develop, through a planned, balanced program of basic and applied research control methods that minimize reliance on extrinsic chemicals. These include investigations of immunological procedures, management practices which minimize exposure of animals to parasitic infections, and natural control agents such as parasites, pathogenic microorganisms, and predators of economically important livestock pests.

USDA PROGRAM

The Department has a continuing long-term program involving veterinarians, parasitologists, pharmacologists, and biochemists engaged in both basic studies and the application of known principles in developing treatments for removal or control of parasites of domestic animals. Research is being conducted on this problem at the following designated locations.

The Federal scientific effort devoted to research in this area totals 13.0 professional man-years. This effort is applied as follows:

Chemical Control of Parasitic Diseases 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

New and Improved Anthelmintics 3.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Hazards of Residues from Treatment for Parasites 3.5 at the Regional Animal Disease Research Laboratory, Auburn, Alabama.

Parasitic and Related Skin Diseases 1.5 at the Albuquerque, New Mexico, field station.

Pathobiology of Parasitic Infections 1.0 at the Albuquerque, New Mexico, field station.

Methods for Control and Eradication of Ticks 1.0 at the Albuquerque, New Mexico, field station.

Control and Eradication of Scabies 1.5 at the Albuquerque, New Mexico field station.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 5.7 professional man-years under the following subheading:

Treatment for Removal or Control of Parasites of Domestic Animals 5.7. Regional research (S-21, Gastrointestinal Parasites of Ruminants and W-35, Nematode Parasites of Ruminants) is providing a scientific basis for chemical control of ruminant parasites. These studies seek to establish efficacy of new compounds against various important parasite species. Methods of administering the compounds and dosages required for most effective parasite control are evaluated along with considerations of toxic effects of the compounds on host animals. Procedures for simplifying the administration of anthelmintics are being developed and their use coordinated with effective management practices. Studies are developing fundamental information on how anthelmintics act upon the metabolic processes of parasites to impair their reproductive processes or result in their death and elimination from infected animals. The problem of drug resistance which has been found to occur in roundworms subjected to prolonged periods of exposure with small amounts of anthelmintics is also being studied. New compounds are being evaluated for use in controlling the spread of trichomoniasis from bulls. Drug dosages, methods of application and frequency of administration are under study. Serological procedures are being studied for possible application in diagnosing the disease.

Industry and Other Organizations, especially chemical companies, are engaged in research programs of varying magnitude designed to discover and develop antiparasitic formulations. These companies have laboratory facilities of their own for preliminary investigations and, to a greater or lesser degree, animal facilities (barns, pens, pastures, etc.) for limited definitive trials. Some of their experimental testing is carried out under contract with State experiment stations and universities; large-scale field trials are occasionally conducted under cooperative agreements with livestock producers. Much of this work is carried out on a confidential basis, the results being unknown to other researchers, veterinarians, and livestock interests. Estimated annual expenditures are equivalent to approximately 110 professional man-years in this broad area which includes livestock in general as well as poultry.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Control of Parasitic Diseases.

In 1961 at the Beltsville Parasitological Laboratory, strains of Eimeria tenella that were serially passed 16 times through groups of chickens receiving nicarbazin, Unistat, Trithiadol, or arsenosobenzene showed varying degrees of resistance to the respective drugs as evidenced by increased severity of cecal lesions, increased oocyst production, and decreased weight gains. Furthermore, deaths occurred occasionally in the groups that were given Trithiadol or Unistat. Drug tolerance was especially pronounced in a strain that was serially passed through 12 groups of nitrofurazone-medicated birds. There was no evidence of cross-resistance, however, when this strain was subsequently tested with Unistat, nicarbazin, arsenosobenzene, glycarbylamide, Trithiadol, and zoalene. These findings were of particular interest because, as previously reported (Cf. 1959 and 1960 Reports), glycarbylamide- and zoalene-resistant strains of E. tenella were also resistant to nitrofurazone.

Studies were continued in sheep with pure infections of Haemonchus contortus (Cf. 1960 Report) to further investigate strain variation in the response of this parasite to phenothiazine. Tests were made with two commercial phenothiazine preparations (purified and N.F. grade products each with an average particle size of about 7 microns) to obtain comparative data on the therapeutic action as well as on the effect of these preparations on egg production and larval development.

Available data at this time suggest that purified phenothiazine may be somewhat more effective than N.F. grades of comparable particle size against resistant strains of Haemonchus. Very little difference in efficacy was noted between these preparations in trials with non-resistant strains of the parasite, each being 99 to 100 percent effective at the 0.25 gram dose level per pound body weight. The purified product, moreover, was completely effective against non-resistant adult worms when given at the rate of 0.125 gram per pound of body weight, whereas the N.F. product at this dose rate was 93 to 98 percent effective. In trials with the Kentucky resistant strain, 0.25-gram dosages of purified and N.F. grade products were 95 and 79 percent effective, respectively; and against the Maryland resistant strain, the corresponding percentage efficacies were 84 and 13 percent, respectively.

In 1962 at the Beltsville Parasitological Laboratory, a strain of Eimeria tenella that had been passed five times through groups of chickens fed 0.00125% amprolium (one tenth the manufacturer's then recommended level) was fully susceptible to the field level of the chemical. This strain has now been passed nine times; from the sixth passage on it has been propagated in birds fed mash containing 0.0025% amprolium. Detectable resistance has developed to this level of the coccidiostat. However, when oocysts recovered from the ninth passage were fed to birds receiving 0.0125% amprolium in the mash, oocyst production was practically nil, gross pathological changes in the ceca were non-existent and there was no detrimental effect on weight gain.

Observations and analysis of the available data suggest that purified phenothiazine may be somewhat more effective than N.F. grades of comparable and larger particle size against resistant strains of Haemonchus contortus. Very little difference in efficacy was noted between preparations of comparable particle size in removing non-resistant strains of the parasites. At dose rates of 0.25 gram per pound body weight, 98 to 100 percent of the adult worms were removed in trials against normally responsive Kentucky and Maryland strains. The purified product, moreover, was 96 to 99 percent effective against both strains at the rate of 0.125 gram per pound body weight, whereas the efficacy of the comparable N.F. product at this dose rate was 88 and 93 percent, respectively. In trials with the resistant Kentucky strain, 0.25-gram dosages of purified and N.F. grade products were 95 and 79 percent effective, respectively; and against the resistant Maryland strain, the corresponding percentage efficacies were 85 and 84 percent, respectively. Similar analyses of the 0.125 gm-level show the purified and N.F. products were 78 and 55 percent effective, respectively, against the resistant Kentucky strain, and 74 and 14 percent effective, respectively, against the resistant Maryland strain. In additional trials with the resistant Maryland strain the purified and N.F. products were 99 and 57 percent effective, respectively, at the 0.5-gram dosage level. The purified chemical at this level was 99 percent effective with the resistant Kentucky strain. Resistance to the N.F. product by the tolerant Maryland strain was further demonstrated at dosage levels of 1.0 and 1.5 gram per pound body weight. Reductions in egg production and larval development followed patterns corresponding to those for the removal of adult worms.

B. New and Improved Anthelmintics.

In 1961 at the Beltsville Parasitological Laboratory, studies showed that Ruelene (Dow M-1261), an organic phosphate, given orally to 2 steers at a dose rate of 60 mg/kg, was highly effective against H. placei, C. punctata, O. radiatum, and T. axei. An emulsifiable formulation (Dow M-1609), poured along the dorsal mid-line of 6 steers at a dose rate of 75 mg/kg, showed some anthelmintic activity; however, its activity was extremely erratic from species to species, and from animal to animal.

Thiabendazole (Merck MK-360) was found to be extremely effective as an anthelmintic in sheep against H. contortus, T. columbriformis, S. papillosus, and Oesophagostomum spp.

An experiment at Poplarville, Mississippi, during 1960 was carried out with 44 head of cattle to test the anthelmintic properties of Ruelene formulation M-1609. The drug was administered by pouring it along the mid-line of the backs of the cattle. The treatment appeared to have some activity against Haemonchus placei, Cooperia spp., and Oesophagostomum radiatum.

In an experiment involving 180 lambs, the anthelmintic activity of phenothiazine, copper sulfate-nicotine sulfate, Ruelene, Co-Ral, and Trivermol was tested. Co-Ral, at a dosage of 7.5 mg/kg of body weight was the most effective in reducing worm numbers, and Ruelene was next best. Trivermol was totally ineffective at the recommended dosage.

Control of over 95% of cattle grubs was obtained treating animals on the 1st of August with an 0.5% Ruelene spray and a Ruelene "Pour-On" formulation at 50 mg/kg, and 75 mg/kg. The animals treated with the "Pour-On" formulation gained more weight than those that were sprayed or left untreated. The peak emergence of grubs in Georgia seems to be around the latter part of January.

An experiment was conducted to compare the efficiency of Ruelene formulation M-1782, with Ruelene formulation M-1560, in the control of gastrointestinal nematodes of sheep. The number of eggs in feces obtained from the sheep was greatly reduced by both formulations, as compared to a control group. The number of eggs being passed by the sheep in the 2 treated groups was much greater than the number from the control group 63 days post-treatment.

Four hundred and eighty lambs, and 520 ewes were treated with 3/4 oz. and 1 oz., respectively, of Ruelene formulation M-2097 (Dow Chemical Company) in a toxicity trial in sheep. One lamb was found dead 24 hours after treatment, but the rest appeared normal until sold 20 days post-treatment.

Two dose levels of Ruelene "Pour-On" formulation M-1609 significantly reduced the number of grubs emerging in the backs of yearlings. The number of nematode eggs in the feces obtained from the treated animals was also significantly reduced, as compared to the control group. However, the low egg counts observed from all animals inject a degree of uncertainty as to the reliability of the results.

Additional critical data were obtained on the anthelmintic action of dithiazanine iodide in dogs and cats. In the aggregate, single dosages of 12.5 mg/kg body weight removed 46% of 52 ascarids from 2 dogs, and 39% of 1,128 hookworms, and 5% of 599 whipworms from 5 dogs. Efficiency against hookworms and whipworms increased markedly when this dosage was given daily for 5 days. In these trials, the chemical removed 60% of 1,581 hookworms, and 80% of 1,141 whipworms from 5 dogs. None of the animals harbored large intestinal roundworms. When the dose rate was increased to 25 mg/kg, and given twice on one day, the chemicals removed 31% of 2,279 hookworms, and 67% of 851 whipworms from 2 dogs. With this dosage also efficiency was substantially increased when treatment was given on 5 successive days. In these trials, the drug removed 72% of 1,678 hookworms, and 85% of 2,539 whipworms from 5 dogs, and all of 28 ascarids from 2 dogs. The dosage of 25 mg/kg, given twice on one day, removed 88% of 42 ascarids from 5 cats. By and large, the several regimens were reasonably well tolerated, although treatment was generally followed by the passage of stained, mucoid feces.

In limited trials with dogs, a new systemic anthelmintic, methyridine, removed 75% of 200 hookworms from 4 dogs, and all of 220 whipworms from 3 dogs. The chemical was given by subcutaneous injection at the rate of 200 mg/kg of body weight. Emesis and signs of transient intestinal discomfort were noted in all of the test animals.

A solution of the organophosphate compound Bayer 13/59 (Dipterex, Dylox) removed 65% of 833 hookworms and all of 259 whipworms when given by intramuscular injection to 2 dogs at the rate of 100 mg/kg of body weight. A similar regimen was completely effective against 12 ascarids in 1 cat, but failed to remove the 2 *Dipylidium* from this animal. One of the dogs vomited shortly after treatment and all animals exhibited a temporary muscular incoordination.

In 1962 at the Beltsville Parasitological Laboratory, thiabendazole, a new anthelmintic for livestock, appeared to be unusually effective against most of the common gastrointestinal parasites of sheep. Thiabendazole, 50 mg/kg body weight, in gelatin capsule, methyridine, 200 mg/kg body weight, by subcutaneous injection; and 2-3 micron purified phenothiazine, 550 mg/kg body weight, as a drench, were studied in comparative trials using flock ewes with moderate to light mixed parasite infections. Preliminary findings indicate marked action against *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Nematodirus*, and *Oesophagostomum*. In one series of trials with flock ewes held on concrete several weeks before treatment, the chemical thiabendazole was adjudged to be about 94 percent effective in removing gastrointestinal parasites compared to 83 percent efficacy, and 45 percent efficacy of purified phenothiazine. Mature *Haemonchus* were recovered from all groups of animals including the thiabendazole-treated ewes. In trials with ewes taken off pasture and treated, thiabendazole was adjudged 99 percent effective, methyridine was adjudged 12 percent effective, and purified phenothiazine was 74 percent effective. Immature *Haemonchus* were recovered from all groups of principals and the controls; mature *Haemonchus* were recovered from the methyridine, phenothiazine, and control groups, but not from the thiabendazole-treated group. No toxicity was observed.

Low-level daily doses of 250 mg. thiabendazole given for 20 days caused a marked reduction in ova per gram feces and numbers of larvae recovered from baermannized cultures of feces. When administration of the drug was discontinued, both egg and larval counts increased but not to pre-treatment levels.

Thiabendazole is highly effective against *H. placei*, *T. axei*, *T. colubriformis*, and *Ostertagia ostertagi*. Its activity is extremely variable against *Cooperia* spp. It is also effective against the fourth-stage larvae of *T. axei*, *T. colubriformis*, *C. punctata*, and *C. pectinata* but not against the third-stage of *O. radiatum*.

Famophos (American Cyanamid Co.), an organophosphate, was found to be a highly effective anthelmintic in cattle against *H. placei*, *T. axei*, *Cooperia* spp., *Oes. radiatum*, and *N. helvetianus*. In sheep it was equally effective against *H. contortus*, *T. axei*, *T. colubriformis*, and *Oes. venulosum*.

An experiment was conducted at Natchez, Mississippi, during the summer of 1961 with 160 lambs to test the following drugs for anthelmintic effectiveness at the dose rates indicated: Ruelene topical application at 75 mg/kg; Co-Ral drench, 5 mg/kg, Co-Ral plus Neguvon drench, 4.5 mg. of Co-Ral and

45.5 mg. of Neguvon per kg. of body weight; Co-Ral topical application at approximately 75 mg/kg; phenothiazine, 25 g. per lamb; Famophos drench, 100 mg/kg; Co-Ral plus phenothiazine drench, 60 mg. of Co-Ral and 25 g. of phenothiazine per lamb; and Promintic, 1 cc. of a 90% v/v aqueous solution per 10 lbs. of body weight. Promintic showed the greatest anthelmintic effectiveness and Co-Ral drench at 5 mg/kg was the next most effective. The topical treatments were the least effective of all treatments, and the other treatments were intermediate in their action against the nematode parasites of the lambs.

Famophos at a dose rate of 50 mg/kg was very effective in cattle against Trichostrongylus axei, and somewhat less effective at 25 mg/kg in a small test in Mississippi. The drug was also effective in grub control, especially at the higher dose rate. Weight gains of the treated animals were as high as untreated controls.

A test conducted to determine the anthelmintic properties of Ronnel (ET-57) in two commercial preparations -- "Rid EZY" Salt at a dose rate of 6.6 mg/kg per day for 30 days, and Ronnel Feed-Mix at a dose rate of 15 mg. per kg. per day for 7 days -- showed no anthelmintic activity as used in this test. Specimens of Trichostrongylus longispicularis were recovered from steers native to northern Mississippi.

The anthelmintic properties of two Ruelene formulations (pour-on and feed additive) were compared in cattle. Both formulations were about 60% effective, as determined by comparing the reduction in the number of worms in treated versus control animals. Pour-on appeared to be more effective for the stomach worms, but the feed additive formulation was more efficacious against the intestinal worms.

C. Hazards of Residues from Treatment for Parasites.

In 1961 at the Auburn, Alabama laboratory, studies showed that lambs weaned when 45 lbs. of weight (early-weaned) had a slightly lower number of internal parasites than lambs which remained with their ewes until the slaughter weight of 90 pounds (late weaned). The difference was significant from the first three slaughters, but it was reversed during the last slaughter. This was probably due to the fact that the lambs remaining on Lot 2 could not adequately graze that pasture, which eventually lost much of its nutritional value.

Tests showed that animals on dry lot harbored a significantly higher number of worms than those on pasture plus various supplements. This was probably due to poor management, as short sward was sparsely distributed throughout the area during most of the test and it was readily consumed by the lambs, thus transmitting the parasites. Contrary to last year's findings, the animals on the pelleted ration had fewer parasites than those on the same diet, but in a mash form, and those on mash supplement had more worms than those on pasture alone.

Experiments have been carried out to determine the effect of feed formulations, in mash and pelleted forms, on worm burdens in rabbits infected with 10,560 to 11,020 T. axei and/or T. colubriformis larvae. Worm counts after 20 days infection have not shown that a marked difference in worm burden exists between animals maintained on mash and animals on pelleted ration.

In 1962 at the Auburn Laboratory, studies showed that contrary to the results obtained during two previous grazing seasons, lambs weaned when 45 lbs. of weight (early-weaned) had a higher average number of internal parasites than lambs which remained with their ewes until slaughtered at about 85 lbs. (late-weaned). The low numbers of parasites encountered and the contradictory results may be explained by the facts that all animals were on pasture for a shorter period (33 days) than during previous years. Besides, the abnormally long winter, followed by summertime conditions without the spring-like transition, produced a sudden abundance of forage.

As a result of an unusually dry fall and long winter, followed by a cold spring restricting the length of the grazing season, parasitism was very low in sheep used in four different nutritional and management programs, consequently, there were no marked differences in numbers of gastrointestinal nematodes.

A non-commercial formulation of mash and pelleted ration had no effect on worm burden in experimentally infected lambs and rabbits. Rabbits maintained on a commercial mash and pelleted ration showed a marked difference in worm burden when experimentally infected with T. axei.

Groups of steers grazed on plots of oats and ryegrass each of which had received different summer treatments - fallowed and a crop of Sudan grass for silage - showed no significant differences between summer treatments and pasture types and pasture loads. The steers on oats harbored fewer Trichostrongylus axei than those on ryegrass, both fallowed and Sudan grass summer crop.

D. Parasitic and Related Skin Diseases.

In 1961, at the Albuquerque, New Mexico laboratory, CO-RAL wettable powder sprays were applied to 3 herds, totalling 215 head of cattle. The spray was applied with a Spray-Dip machine to 2 herds, and with a power sprayer to the third herd, delivering the spray at about 400 psi. Ninety-eight percent control of the grubs was obtained with the Spray-Dip machine and 93 percent with the power sprayer. Ruelene emulsifiable sprays, having a concentration of active ingredient of 0.375 percent, was applied to 2 herds composed of 298 head. No control was obtained with the power sprayer, but 84 percent control was obtained with the Spray-Dip machine. No satisfactory explanation is offered for the one complete failure to control cattle grubs with Ruelene at 0.375 percent. Preliminary tests with Famophis, an injectible organic phosphate compound, for the control of larvae of the sheep nosebot, Oestrus ovis, showed very promising results. It was determined to be 100 percent effective biologically against first instars, and 95 percent effective

against all three larval stages. Furthermore, Famophos was found to possess a wide margin of safety.

One hundred seventy-two sheep were examined for incidence of infestation with larvae of the sheep nosebot, Oestrus ovis. Only 17 were free of parasites. The infestations were found to be made up of first, second, and third instars, in percentages of 79, 13, and 7, respectively. Single annual treatment of a flock of isolated range sheep with injectible dimethoate for the control of Oestrus ovis started in 1958, show interesting results. Before treatment in the fall of 1958, the flock was determined to be 100 percent infested, with an average of about 59 larvae per animal. In 1959, the average number of larvae was reduced to an average of 26 per head. In 1960 a slight increase in the average number of larvae per head was recorded, and was attributed to the fact that the entire flock was not treated. In fact, only about 28 percent of the animals were treated.

Dimethoate for the control of Oestrus ovis larvae infesting sheep was found to be less effective when administered subcutaneously as compared with the intramuscular route. Although the dosage administered subcutaneously was about 55 percent greater, the reduction in average number of larvae and the reduction in the percent of infested animals was less than when a lower dose was administered intramuscularly. In a limited but controlled test, it was found that dosages of dimethoate, ranging from 16 - 18.9 mg/kg were 100 percent effective against first instars of Oestrus ovis. Field tests with injectible dimethoate show that sheep under New Mexico range conditions cannot tolerate dosages of 25 mg/kg. They can be treated safely with 20 mg/kg. Nearly 100 percent effectiveness is attained against first instars.

Low level feeding of ronnel, an organic phosphate insecticide developed for the control of cattle grubs and other livestock ectoparasites, was found ineffective against psoroptic scab mites of sheep. Dosage levels of 5 mg/kg a day for 30 days, and 10 mg/kg for 20 days, had little or no effect on the mites.

In 1962 at the Albuquerque, New Mexico, laboratory, tests to control cattle grubs, Hypoderma bovis and H. lineatum, showed that very high levels of control could be obtained with 0.25 percent CO-RAL applied with the Spray-Dip machine. An interesting feature of the treatment was the use of the grub rake, which apparently enhanced the effectiveness of CO-RAL. Tests in New Mexico with Bayer 29493 showed that at a concentration of 0.15 percent, it was as effective as CO-RAL at 0.5 percent, when applied as a spray.

Dimethoate for the treatment of sheep infested with Oestrus ovis was toxic to the sheep at 13 - 25 mg/kg, despite all measures taken to prevent intoxication. Therefore, this highly effective compound cannot be recommended for treatment of this parasite under range conditions.

Tests with Famophos for the control of Oestrus ovis show considerable promise as a treatment for sheep infested with this parasite. No toxicity was encountered at dosages of from 40 - 70 mg/kg. More than 95 percent effectiveness against first instars was obtained with doses of approximately 50 mg/kg. More precise data on the effectiveness of Famophos against second and third instars will likely be obtained if evaluation of the results is made a week after treatment rather than 72 hours as has been done with dimethoate.

Topical application of CO-RAL, Bayer 29493, and Famophos for the control of O. ovis indicates that 97 percent control can be obtained with Bayer 29493. The other two compounds were ineffective. The need for additional tests with this compound and the method of application is indicated.

Biological tests with O. ovis larvae show that third instars can be kept alive for as long as 62 days, providing bacterial contamination is kept at a minimum, and the temperature at about 10°C. Second instars were kept alive 49 days under similar conditions. Larvae food requirements, optimum conditions of temperature and RH have as yet not been determined.

E. Pathobiology of Parasitic Infections.

In 1961 at the Albuquerque, New Mexico, laboratory, low level administration of ronnel, an organic phosphate compound recommended for the control of cattle grubs and other livestock ectoparasites, was found to be ineffective against the blood sucking louse of cattle, Hematopinus eurysternus. Dosage levels of 5 mg/kg/day for 27 days and 10 mg/kg/day for 22 days, were found to be ineffective in controlling lice on louse-carrier animals.

Aqueous sprays of CO-RAL having a concentration of 0.1 percent were found to eradicate an infestation of Damalinia bovis in a herd of 24 calves, after a single spraying applied with a Spray-Dip machine. This same formulation was found just as effective on 6 calves infested with the blood sucking louse, Linognathus vituli. It was used on one laboratory cow heavily infested with Hematopinus eurysternus, and no lice were found for a period of 40 days after treatment. At this time the results were somewhat complicated by the climatological conditions which would have brought about a natural reduction in the louse population, so no statement regarding eradication is being made. One-tenth percent aqueous spray of CO-RAL was also very effective in treating 3 swine infested with the hog louse, Hematopinus adjecticius. No lice were found on these animals over a period of 65 days, which is considered to be virtually eradication.

Malathion emulsifiable concentrate spray of 0.5 percent appears to have eradicated a louse population on a "carrier" animal, when treated in the spring. The date of treatment was March 22, 1961. A replication of this test is needed in the fall or early winter to determine if eradication can actually be achieved. Treatments for lice applied in the spring are difficult to assess because of the natural population reduction that accompanies the advent of warm weather.

A herd of range Herefords, known to have been heavily infested with lice, Hematopinus eurysternus, in the winter, were sprayed in June 1960, with 0.375 percent Ruelene prepared from an emulsified concentrate. Lice were found 14 days after treatment on certain indicator animals, so the herd was re-sprayed, using 0.5 percent toxaphene emulsified concentrate spray. The cattle were examined about 6 months later (December 13, 1960) and no lice were found. They were again examined on January 18, 1961, and a total of 5 lice were found on 2 bulls. This was interpreted to mean that excellent control, but not eradication can be achieved by two sprayings, 14 days apart, using Ruelene and toxaphene sprays prepared from emulsified concentrates, if applied in the summer when the louse population is reduced by natural conditions, such as warm weather and the resulting loss of winter hair coat.

Field tests for the elimination of louse infestations in 2,000 head of cattle in Virginia were continued this fiscal year. This herd was originally infested with 4 species of lice in the spring of 1957, when the tests were started. At this time, the cattle were sprayed twice with lindane, applied with a Spray-Dip machine. In 1958, the treatment was modified by substituting malathion for lindane. The application of these treatments apparently eliminated Damalinia bovis and Solenopotes capillatus, but not Hematopinus eurysternus nor Linognathus vituli. In 1959 and 1960, the treatment regimen was again modified by using rotenone suspensions followed in 2 weeks by malathion. During the examination of the cattle in April 1961, L. vituli was present, as it has been through the tests; H. eurysternus absent, D. bovis absent, and S. capillatus present but in small numbers.

In 1962, at the Albuquerque laboratory, low level feeding of ronnel to cattle and sheep infested with psoroptic mites, and cattle infested with biting and blood-sucking lice, appeared to satisfactorily reduce louse populations when administered at the rate of 20 mg/kg/day for 34 days. Its effect on psoroptic mite infestations was less satisfactory. The indications are that this method of parasite control is less effective and economical than dipping or spraying.

Successful control of cattle lice in a herd of 2,500 cattle in Russell County, Virginia, was obtained with 0.25 percent CO-RAL WP spray, applied with a Spray-Dip machine. From the standpoint of biological efficiency and cost it was determined that one spraying with CO-RAL was not superior to two treatments with malathion applied at intervals of 10-12 days.

F. Methods for Control and Eradication of Ticks.

In 1961 and 1962 at the Albuquerque, New Mexico, station, preliminary dipping tests with insecticides for the control of ticks (Boophilus annulatus and B. microplus) infesting cattle in Mexico showed that satisfactory control could be obtained with a single treatment of CO-RAL, at concentrations of 0.25, 0.12, and 0.06 percent. Even at 14 days after dipping, 100 percent mortality was evident in cattle dipped in 0.25 percent concentration. Korlan was not as effective. Seven days after dipping only 50 percent mortality was obtained with 0.5 percent concentration. The animals were re-dipped and effective control was obtained. Delnav virtually eradicated infestations in 7 days, thus making it one of the most effective compounds used.

G. Control and Eradication of Scabies.

In 1961 at the Albuquerque, New Mexico, laboratory, small scale dipping tests for the control of psoroptic scab in sheep with several compounds classified as acaricides or acaricide-insecticides, were conducted at the Animal Disease and Parasite Research Laboratory at Albuquerque, New Mexico. To prove the effectiveness of the compounds, the treated animals were held for examination and observation for long periods, lasting in some instances for a year. The conclusions drawn from these tests were that none of the compounds, with the possible exception of 0.5 percent CO-RAL, was as, or more effective than Lindane, taken as a standard. The compounds tested were malathion, Tedion, Korlan, CO-RAL, Dylox, Bayer 22,408, Bayer 28,589, Bayer 30,686, Bercotox, Orthorix, Triethanolomine, and Sevin. Sheep were used in all but 2 tests. Sevin was used on a calf heavily infested with psoroptic mites, and 0.5 percent CO-RAL was used on swine infested with sarcoptic mites.

Attempts to transfer psoroptic mites infesting ears of goats to the ears and bodies of sheep failed insofar as it was not possible to produce infestations on sheep that would produce sequential generations of mites creating permanent infestations. Transfer of mites from goats to sheep were made manually, and attempts to transfer them naturally were made by keeping infested goats and clean sheep penned together. Some of the penned animals were kept together for more than a year without producing psoroptic scabies in the sheep.

Eighteen head of cattle heavily infested with psoroptic mites were sprayed with CO-RAL, using a Spray-Dip machine. Three animals were used as untreated controls. The aqueous suspension sprays used had concentrations of 0.125, 0.25, and 0.5 percent. After treatment the animals were isolated for observation and examination in groups based upon the spray concentrations used. At this date it is too early to assess the results. The animals will be kept under these conditions for additional examinations and observation throughout the coming summer, fall and winter.

Detailed examination of 11 sheep in June, when the winter-time lesions were completely resolved, was negative for the presence of live mites. Although such hiding places as inguinal fossae, ears, infra-orbital glands and interdigital spaces were searched for mites, none were found. Despite the negative results, it is believed that a few individual sheep, going through the latent phase of scabies infestation, harbor sufficient mites over the summer months to form a nucleus of infection for the flock during the fall and winter when optimum conditions for the development prevail.

In Illinois, 8 toxaphene tests were started for the control of psoroptic sheep scab in 1959. These tests were carried through the fall, winter, and spring seasons of 1959-60. Observations on the treated flocks covered periods of from 3 to 18 months, and show that toxaphene dips, 0/5 percent concentration of active ingredient, applied once were highly effective in the control of psoroptic sheep scabies. Following these tests, 395 flocks, totalling 22,053 head, were treated by dipping in 0.5 percent toxaphene by state and Federal Livestock Inspectors in cooperation with the Animal Disease

and Parasite Research Division. The results showed that of the 395 flocks 11, or approximately 3 percent, subsequently showed clinical evidence of scabies, and required re-treatment. Live mites were found on 5 of the 11 flocks. Failure to control scabies in these flocks could not always be attributed to the treatment because reinfestation from untreated sheep was a possibility that could not be eliminated. The conclusions drawn were that 0.5 percent toxaphene, applied as dips to sheep infested with psoroptic mites under a variety of conditions, was a safe and effective method of controlling psoroptic sheep scab.

Ethion, an organic thiophosphate developed as a miticide, was found to be ineffective against psoroptic mites infesting sheep when used as dips having 0.1, 0.5, and 0.75 percent concentration of the active ingredient. Furthermore, the compound was found toxic to lambs and goats, but not to ewes at concentration levels of 0.75 and 0.5 percent.

In 1962, at the Albuquerque, New Mexico, laboratory, dipping tests with candidate compounds for sheep scabies (Psoroptes ovis) indicate that Malathion 0.5%, CO-RAL 0.25%, Diazinon 0.05%, Shell 4294 0.2%, and possibly Delnav, show the most promise as effective miticides. Additional tests using larger groups of animals are needed. CO-RAL at 0.25% spray for cattle scabies (Psoroptes bovis) shows promise as a means of controlling this parasite.

Tests to provide information on the protective period of lindane and toxaphene against infestation with P. ovis indicate these two compounds can provide protection under maximum mite population pressure for approximately 72 days.

Tests to determine the rate of depletion of gamma isomer in sheep dipping vats appear to have raised more questions than answers. Data collected indicate that depletion in dipping vats is probably due to selective removal by the wool of sheep rather than aging or contamination of vat samples.

A survey of the incidence of Chorioptes ovis in Southwestern sheep shows this parasite to be far more prevalent than usually believed. The significance of this finding is hard to interpret because in only 1 animal were mites associated with lesions.

Forty heads of sheep going through the latent phase of psoroptic scabies were examined carefully for mites. Although the body areas known to have had active lesions during the winter and early spring were carefully examined, no mites were found on these areas. The likely body hiding places, such as the ears, nares, infraorbital, inguinal and interdigital spaces were carefully searched for mites but none were found. Later, with the advent of cooler weather, scabies infestation on these animals became apparent, and 6 weeks after the first symptoms were noted, the flock was 100 percent infested. This is taken to mean that latent scabies infestation in sheep can go undetected during the hot summer months in New Mexico. The nature of the infestation's survival is not presently very well understood.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

- Allen, R. W., F. D. Enzie, and K. S. Samson. 1962. The Effects of Bithionol and Other Compounds on the Fringed Tapeworm, Thysanosoma actinoides of Sheep. Amer. J. Vet. Res., 23:236-240.
- Baird, D. M., H. C. McCampbell, H. Ciordia, W. E. Bizzell, and P. E. White. 1961. Effect of two systemic insecticides for the control of cattle grubs. J. An. Sci. 20:388.
- Ciordia, Honorigo, William E. Bizzell, and D. M. Baird. 1961. The Efficacy of Two Formulations of an Organo-Phosphate in the Control of Gastrointestinal parasites of sheep. Jour. An. Sci., 20:388.
- Ciordia, H., W. E. Bizzell, D. M. Baird, and H. C. McCampbell. 1962. Effect of Weaning Time on Parasitism in Lambs. Jour. An. Sci., 21:384-385.
- Colglazier, M. L., and F. D. Enzie. 1960. Comparative trials with some present-day anthelmintics for swine. J. Parasit. 46:808.
- Colglazier, M. L., F. D. Enzie, and E. H. Wilkens. 1960. Some chemotherapeutic trials in canine demodectic mange. Proc. Helm. Soc. Wash., 27:139-145.
- Colglazier, M. L., and F. D. Enzie. 1960. Anthelmintic trials with hygromycin in pigs. J. Parasit. 46:796.
- Colglazier, M. L., and F. D. Enzie. 1961. Treatment of experimental lungworm infections in calves and pigs with cyanacethydrazide. Proc. Helm. Soc. Wash., 28:96-91.
- Enzie, F. D., and M. L. Colglazier. 1960. Teniacidal trials with some diphenyl sulfones in cats, dogs, and chickens. Amer. J. Vet. Res., 21:624-627.
- Enzie, F. D., M. L. Colglazier, G. E. Whitmore, and D. E. Thompson. 1960. Effectiveness of three management systems on parasitism in lambs. III. Resistance of Haemonchus contortus to phenothiazine. J. Parasit. 46:41.
- Enzie, F. D., and M. L. Colglazier. 1960. Preliminary trials with bithionol against tapeworm infections in cats, dogs, sheep, and chickens. Amer. J. Vet. Res., 21:628-630.
- Enzie, F. D. 1961. Treatment and Control of Gastrointestinal Nematodes. The Nat'l Wool Grower, 51:21.
- Foster, A. O., J. R. Douglas, A. C. Todd, and F. D. Enzie. 1961. The Status and Use of Phenothiazine in Cattle. Amer. Vet. Med. Assoc. 139:490-491.
- Herlich, Harry, Dale A. Porter, and Robert S. Isenstein. 1961. Critical Tests on the Anthelmintic activity of Ruelene administered to cattle orally and topically. Vet. Med., 56:219-221.

Herlich, Harry. 1962. Observations on the efficacy of Thiabendazole, Ruelene and Phenothiazine as an Anthelmintic in Ruminants. Jour. Parasit. 48(2 Sec.2):29.

Kates, K. C., J. H. Turner, I. Lindahl, G. E. Whitmore, and F. D. Enzie. 1960. Effectiveness of three management systems on parasitism in lambs. I. Clinical effects of parasitisms relative to exposure and medication. J. Parasit. 46:40.

Knight, Robert A., John A. McGuire, and Louis B. Walton. 1960. Mississippi tests on a new anthelmintic in sheep. Vet. Med., 55:71-74.

McLoughlin, D. K., J. L. Gardiner, and D. K. Chester. 1960. The activity of glycarbylamide, Trithiadol and nicarbazin against Eimeria tenella in chickens. Poultry Sci., 39:1329-1332.

McLoughlin, D. K., and J. L. Gardiner. 1961. Drug-resistance in Eimeria tenella. I. The Development of a Glycarbylamide-resistant Strain. J. Parasit., 47:1001-1006.

McLoughlin, D. K., and J. L. Gardiner. 1961. Zoalene Tolerance by Eimeria tenella. J. Parasit. 47(4, Sect.2):46.

McLoughlin, D. K., and J. L. Gardiner. 1962. The Activity of Amprolium in Eimeria tenella Infections - Laboratory Trials. Avian. Diseases, 6:185-190.

Peterson, H. O., W. P. Meleney, and N. G. Cobbett. 1960. The Use of Organic Phosphorus Compounds in Destroying Oestrus ovis Larvae. Proc. 64th. Ann. Meet. U.S. Livestock San. Assn. pp. 178-186.

Turner, J. H., K. C. Kates, I. Lindahl, G. E. Whitmore, and F. D. Enzie. 1960. Effectiveness of three management systems on parasitism in lambs. II. Kinds and levels of parasitisms relative to exposure, medication, and weather. J. Parasit., 46:40.

MISCELLANEOUS PARASITES AND PARASITIC DISEASES
Animal Disease and Parasite Research Div., ARS

Problem. Parasitism is a way of life that characterizes the majority of living things. Except for basic life processes, it is probably the commonest biological phenomenon. More than 50,000 kinds of animal parasites (i.e., parasites classified as animals as opposed to those classified as plants) are known. New varieties are being discovered and described at a rate of about 500 per year. Some devastating parasites, indigenous to foreign countries, threaten to surmount barriers imposed against them. Certain of these have already gained new footholds in livestock, poultry, and wild-life. Essential elements of procedure against parasites--established, exotic, or new--are accurate diagnosis, development of full knowledge about them, and research on effective control measures. The primary requirement is development through research of up-to-date knowledge of classification and identification supported by a complete reference collection of parasites, including type specimens and familiarity with global research already done. Basic investigations of parasitisms as biological phenomena are involved, especially in host-parasite relations, immunology, serology, ultrastructure, and other aspects of diagnosis and control. The problem is to develop and maintain up-to-date methods of identification and the essential, supporting reference collections, as well as complete parasitological information extracted from the world's scientific literature; investigate important phenomena and host-parasite systems not covered in specific host categories; and provide bases for detection and control that are adequate to meet existing and anticipated needs, through research on problems involving various parasites and hosts, including wild animals and birds important to agriculture.

USDA PROGRAM

The Department has a continuing long-term program for parasitologists, biochemists, and microbiologists, engaged in basic and applied research in this area. Research is being conducted on the following problems at the designated locations.

The Federal scientific effort devoted to research in this area totals 11 professional man years. This effort is divided among subheadings as follows:

Classification of parasites 2.0 at the Beltsville Parasitological Laboratory.

Maintenance of parasite collection 1.0 at the Beltsville Parasitological Laboratory.

Maintenance and publication of author, subject, and host index-catalogues 2.5 at the Beltsville Parasitological Laboratory.

Incidence of livestock parasites in the Southeast 0.5 at Tifton, Georgia.

Immunologic and other biologic approaches to the prevention and control of parasitic diseases 5.0 at the Beltsville Parasitological Laboratory.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

Work with miscellaneous parasites and parasitic diseases has no counterpart in the States. Other than in USDA, no complete parasite collections or indices of parasitological information are being developed and maintained in the United States. The USDA is almost the sole source of comprehensive information on identification, distribution, and economic importance of parasites and up-to-date treatment. Such information is furnished to workers in industrial concerns, experiment stations, colleges and universities, Federal and private research laboratories, and the Armed Forces.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Classification of parasites.

In 1961 at the Beltsville Parasitological Laboratory, two hundred and ninety-four lots of specimens were received for identification (95 nematodes, 31 cestodes, 22 trematodes, 42 arthropods, and 4 lots of miscellaneous material). Of particular interest was the identification of Rhipicephalus evertsi evertsi, the red tick, from an African antelope (eland) exhibited in an animal compound known as "Africa U.S.A." at Boca Raton, Florida. On further examination other animals in this zoo-like compound were found to be harboring the red tick. The red tick can transmit Theileria parva (the cause of East Coast fever), T. mutans, Babesia bigemina, B. equi, B. caballi, Rickettsia ruminantium (the cause of heart-water), R. conorii, and Borellia theileri. Three new helminths and a protozoan parasite were described in 6 papers published during the year.

In 1962, two hundred and seventy-seven lots of specimens were identified during the fiscal year (36 cestodes, 137 nematodes, 27 trematodes, 73 arthropods, 3 protozoans, and 1 lot of miscellaneous specimens). Among the cestodes were 3 Sparganum, an immature stage of the genus Spirometra, from the muscles of swine in Florida. This parasite is of human medical importance. Although reported in swine from other parts of the world, it has not been previously reported from swine in the United States. Observed inside one of the Sparganum was an Agamodistomum, an immature stage of a trematode, that also occurs in the muscles of swine.

Dracunculus insignis, a species very similar to one occurring in man in oriental and middle eastern countries has been received from dogs in Virginia and Florida, from a skunk in Maine, and from raccoons in Maryland. Specimens from the opossum, tentatively identified as D. fuelleborni, were received from Aberdeen, Maryland. Previous reports of this nematode in America are comparatively rare.

The tropical horse tick, Dermacentor nitens, has become established in Florida, having been found in several counties. Recently we received ticks for identification taken from a sick horse. The illness was diagnosed as piroplasmosis. The ticks removed from the sick animal were determined as Dermacentor nitens, the tropical horse tick. Previous to 1960 this tick was not known to occur in Florida.

B. Maintenance of parasite collection.

In 1961, at the Beltsville Parasitological Laboratory, five hundred and forty-five lots of specimens were catalogued in the Parasite Collection of the Laboratory - 384 were recorded in the Animal Parasite Collection and 161 in the U. S. National Museum Helminthological Collection.

Four hundred and forty-five inquiries and requests for information were handled during the period of this report. This covered the following subjects: Identification of specimens - 155; loan of specimens - 76; cataloguing of specimens - 53; manuscripts - 100, and miscellaneous - 61.

Four parasitologists from universities in the United States and Canada stayed from 1 to 8 weeks at the Laboratory to study specimens.

Approximately 17,000 specimen jars were checked for loss of preserving fluid and corrosion of covers. Fluid was added to, or covers replaced, on 75 per cent of the specimen jars.

In 1962 a total of 1,750 lots of specimens composed of 117 cestodes, 574 nematodes, 883 trematodes, 164 arthropods, 5 protozoans, and 7 miscellaneous organisms, were assigned catalogue numbers, location numbers, and recorded in the parasite and host indexes of the Collection. This makes them immediately available for study and comparison with new specimens received for identification. Approximately two-thirds of the specimens recorded represent part of a large backlog of parasites that were received during preceding years for deposit in the Collection. Among the old specimens were parasites collected around 1890 from Australian domestic and wild animals, marine fish, American wildlife, and ticks used in experimental transmission of anaplasmosis.

In connection with maintaining the Collection, approximately 440 inquiries were answered during the year. The inquiries covered the following subject matter: Identification of specimens 138; loan of specimens 67; cataloguing of specimens 47; nomenclature 14; manuscripts 76, and miscellaneous 98.

During August and September 1961, the Collection was moved from the basement of Bldg. 318 to the basement of Bldg. 120, a distance of about 3 miles. This was accomplished without breakage or loss of specimens.

During the year numerous requests for identification of nematodes, acanthocephalans and linguatulids have been received from 22 States, including Alaska, Hawaii and the Canal Zone, as well as from Africa, Pakistan, Formosa, South America, Egypt and the Azores.

C. Maintenance and publication of author, subject, and host index-catalogs.

In 1961, at the Beltsville Parasitological Laboratory, the Index-Catalogue of Medical and Veterinary Zoology was maintained and expanded in its various sections: Author, Subject, and Host Catalogues, and Checklist of Specific and Subspecific Names. New entries augmenting the Catalogues were as follows: Author entries, 9,958; Parasite entries, 21,224; and Host entries, 7,557. The Index-Catalogue has continued to supply references for the Treatment Catalogue of the Antiparasitic Investigations Research Group and to index literature on plant parasitic nematodes. Added to the Host Catalogue have been 343 genera, 1,213 species and 421 subspecies previously unrecorded as hosts. This growth reflects in particular increased material on parasites of fishes and insects.

New genera and species of parasites were as follows:

	<u>New genera</u>	<u>New species</u>
Protozoa	20	146
Trematoda	45	293
Cestoda	8	76
Nematoda	49	279
Arthropoda and misc.	<u>110</u>	<u>397</u>
Total	232	1,201

The Index-Catalogue has had approximately 103 visits by specialists of the United States, and at least 11 other countries, using it as a source of information. Two parasitologists from Japan were consulting it for the entire year.

In 1962, new entries augmenting the Catalogues were as follows: Author entries, 7,782, Parasite entries, 13,598, and Host entries, 4,240. The Index-Catalogue has continued to supply references for the Treatment Catalogue of the Antiparasitic Investigations Section and to index literature on plant parasitic nematodes.

New genera and species of parasites are as follows:

	<u>New genera</u>	<u>New species</u>
Protozoa	7	203
Trematoda	43	331
Cestoda	12	79
Nematoda	22	276
Arthropoda and misc.	<u>23</u>	<u>411</u>
Total	107	1,300

The Index-Catalogue has had approximately 120 visits by specialists of the United States and at least 15 other countries, using it as a source of information. Two parasitologists from Japan visited the Index-Catalogue from July 1, 1961, to May 25, 1962, and used it as their source for material for publication of monographs on parasites.

D. Incidence of livestock parasites in the Southeast.

In 1961, at the ADP Division Laboratory at Tifton, Georgia, 43 postmortem examinations were made of pigs and two tissue samples were examined for parasites. Twenty of these cases had parasite worm counts high enough to consider parasitism as the prime factor causing unthriftiness, scouring, etc. Twelve of these involved predominantly Strongyloides ransomi, three Ascaris suum, three of both of these species, and one involving these, plus Oesophagostomum sp. One of the tissue sections (small intestine) was heavily parasitized with Strongyloides ransomi and the other tissues examined contained Physocephalus sp.

For the third year the single most important parasite in pigs was Strongyloides ransomi. This has become particularly important in baby pigs which are becoming infected prior to birth. Adult worms have been recovered from the small intestines of baby pigs only 2 days old. These pigs were scouring from the time of birth. One 4-month-old pig harbored 1,288 Ascaris suum, all of which were young, immature forms. These were recovered from the oesophagus, stomach, small and large intestines, liver and gall bladder. Thirty fecal samples were examined. Sixteen were positive and 14 were negative. Strongyloides ransomi eggs were present in moderate to high counts in all 16 cases, Ascaris suum in one case, and Oesophagostomum sp. in three cases.

Twenty-nine cattle fecal samples were examined during the year for evidence of parasitism. Twenty-one were positive and eight were negative. Of the positive cases, only three had high enough egg counts to be considered of possible clinical significance. These were in three young dairy calves which were passing eggs of Strongyloides papillosus. Fourteen postmortem cases were examined for parasites and all but one were positive. Eight of these had severe damage to the stomachs and the predominant causative agent in seven was determined to be Ostertagia ostertagi. The eighth case showed the general appearance of "bottle jaw" and the stomach was extremely edematous and had many necrotic ulcers that appeared healed. Approximately 300 O. ostertagi were recovered, but no Haemonchus placei which usually causes this condition.

Two cases of particular interest were from a 5-year-old, and a 9-year-old brood cow which had severe infections of O. ostertagi. The two cows were from adjacent farms and were brought to the laboratory on the same day. The stomachs of both were slightly edematous and the mucosal surface was covered entirely with small fibrous nodules and tiny petechial and minute haemorrhagic ulcers. The 5-year-old cow had 1,120,000 O. ostertagi and the 9-year-old cow had 878,000 O. ostertagi.

Haemonchus placei was recovered from three clinical cases, Cooperia punctata from four, and Trichostrongylus axei from two cases.

Twenty-seven fecal samples from horses were examined. Twenty-four were positive for strongyle larvae, but all in low numbers.

Twenty-eight fecal samples from dogs were examined. Twenty-three were positive and the eggs indicated hookworm as the predominant parasite present.

This project was discontinued November 3, 1961.

E. Immunologic and other biologic approaches to the prevention and control of parasitic diseases.

In 1961 at the Beltsville Parasitological Laboratory, studies were continued on the cultivation of Oesophagostomum radiatum in a medium containing principally trypticase, yeast extract, glucose, cattle serum and extracts of rabbit embryo and pig liver, and in modifications formulated by the addition or deletion of ingredients. Cultures were examined at 1-to 4-day intervals during an incubation period of 24 days at 38.5 C.

Preliminary results on the rate of development in 8 variations indicated that all ingredients of the original medium are essential except swine liver extract. Indeed, larvae grown in media without this constituent equaled or surpassed the rate of development of those grown in its presence. Larvae cultured in the absence of serum showed little or no growth after 24 days incubation. When certain substitutes were added in place of serum, growth and development occurred but was delayed.

Hyostrongylus rubidus, Oesophagostomum quadrispinulatum, Oes. radiatum, Hamonchus contortus (bovine), Ostertagia ostertagi, and Cooperia spp. were cultured to fourth stage in a medium containing principally trypticase, yeast extract, glucose, sheep or cattle serum, and extracts of rabbit embryo and pig liver. Two ovine strains of H. contortus, one resistant and the other non-resistant to phenothiazine, reached fourth molt in the same medium. Addition of a vitamin supplement to some of the cultures accelerated development, but in vitro growth of all species was slower than that in vivo.

The influence of tissue extracts and sera from various sources on the growth of Oesophagostomum radiatum was studied by adding these materials to a medium comprised largely of trypticase, yeast extract, glucose, and extracts of rabbit embryo and pig liver. Extracts of the small intestine of a helminth-free calf (C-1) and serum from a helminth-free yearling (C-2) had little effect on development to early fourth stage. A distilled water extract of the intestinal mucus and ingesta from C-2 and from C-3 (yearling patent with Oes. radiatum) accelerated growth markedly while that from C-4 (yearling resistant to Oes. radiatum) had a lesser effect. A definite lag in development occurred in media containing sera and extracts of the cecum and colon from C-3 and C-4. In all cultures containing tissue extracts of C-3 and C-4, most larvae had precipitates at the body openings and were covered with a coating of media debris.

Studies were continued on the evaluation of the agar diffusion precipitin test as a method of diagnosing prepatent kidney worm infections in swine. Sera obtained from swine in a program for management control at Tifton, Georgia, were tested as were sera obtained from kidney worm-free herds at Beltsville, and from swine infected experimentally. Results indicate that the method is reliable, even for low-level infections, when tests are made on a number of consecutive serum samples.

Analytical studies revealed little evidence of urea or uric acid in extracts of the excretory glands of the swine kidney worm (Stephanurus dentatus). They showed, however, the presence of proteins, some of which proved to be antigenic in the agar diffusion precipitin test for the diagnosis of kidney worm infections. Compared to other parts of the worm body, the excretory gland contained a high proportion of the soluble proteins and these showed most of the precipitinogenic activity. In addition to the excretory glands, the esophagus, head, and intestine gave strong precipitin lines. The body fluid gave very weak lines, and the cuticle and gonads were negative.

In limited trials, the number of Oesophagostomum radiatum, Ostertagia ostertagi, and Cooperia oncophora, developing to adult males in susceptible hosts, was inversely proportional to x-ray doses ranging from 10,000 to 22,800 roentgens administered to the infective larvae. Moreover, the total number of Ostertagia and Cooperia that became established was significantly reduced as compared to a control which received non-irradiated larvae. Although egg counts were reduced and some abnormal ova were observed, the F₁ generation of O. radiatum produced an apparently "normal" infection in a susceptible host.

In 1962, at the Beltsville Parasitological Laboratory, phosphate buffer extracts of organs and tissues of Stephanurus dentatus, which had previously been shown to contain specific precipitinogens, were injected into swine in an attempt to produce immunity to subsequent challenge. One, 2, or 3 injections, spaced 1 week apart, were followed by challenge with 1,000 infective kidney worm larvae given 7, 14, 21, or 28 days after the last injection. Principals and controls challenged at the same time were necropsied 28 to 30 days later, and the degree of infection graded by the number and gross appearance of the liver lesions.

Partial protection was demonstrated when single injections of either excretory gland extract or intestinal tissue extract or a combination of both were followed by challenge after 14 days. Swine challenged before or after this period were comparable to controls in most cases and occasionally showed greater pathology. Multiple injections did not provide a greater degree of protection than single injections regardless of the time interval before challenge.

Stephanurus dentatus developed to fourth stage in 15 days in a complex medium (SM-1) containing principally trypticase, yeast extract, glucose, extracts of rabbit embryo and pig liver, and 10 percent cattle serum. Identical development was also achieved in 3 modifications formulated by the addition of: double amounts of swine liver extract and bovine serum; a double amount of swine liver extract; and a double amount of autoclaved swine liver extract. In Pitts' medium, which differs principally from SM-1 and its modifications by an increased amount of bovine serum (25 percent) and the absence of tissue extracts, developmental time to fourth stage was reduced.

Studies on the effect of ionizing radiation on the infective larvae of Oesophagostomum radiatum were continued. As previously reported a selective lethal action on males was demonstrated which could be correlated with the total dosage. The presence of vaginal plugs as an evidence of fertilization of female worms was adopted as a criterion for estimating male development after irradiation. At a dose of 9,000 r most males reached maturity, mated, and died before necropsy of the host. However, at a dose of 13,000 r most males died before reaching maturity.

Cooperia punctata was cultured from artificially exsheathed third-stage larvae to immature adults in a medium containing principally trypticase, yeast extract, glucose, bovine serum, and extracts of rabbit embryo and pig liver. Similar development was obtained in modifications formulated by the addition of intestinal tissue extracts from bovines parasitized with Oesophagostomum radiatum or extracts of rumen contents, ingesta and mucus, or intestinal tissue from a calf parasitized with C. punctata. Fifth-stage was reached in all media in 13 to 19 days but survival, particularly after reaching fourth-stage, was poor.

Antibodies were demonstrated in the serum of a calf infected with Cooperia punctata by using living infective third-stage larvae and in vitro grown parasitic larvae of this nematode as sources of antigen. The visible reactions, larval precipitates and cuticular coating, which were strong during patency and weak after patency had ended, became very strong 4 days after challenge with C. punctata and remained strong up to 50 days after challenge.

Using "immune" serum in an agar double diffusion technique, precipitinogens were demonstrated in extracts of adult Oesophagostomum radiatum collected from bovines but not in extracts of larvae grown in vitro. Stronger reactions were obtained with the serum from a multiply infected calf than with serum from a singly infected calf.

Ultracentrifugation through sucrose gradients of swine serums, showed the absence of serologically-active macroglobulins in serums from swine infected with kidney worms as well as from noninfected swine; antibodies to kidney worm antigens in extracts of kidney worm excretory glands sedimented slowly while specific antigens sedimented more rapidly, indicating the possibility of separating nonspecific and specific antigens by this method. The main components of extracts of kidney worm excretory glands and intestines were found to have sedimentation coefficients of 3.6 S and 13.5 S, and 2.8 S and 12.2 S, respectively.

Absorption studies failed to prove conclusively the occurrence of common antibodies to kidney worm antigens in serums from infected swine and in serums from noninfected swine.

Electrophoresis in agar indicated that nonspecific and specific antigens of the swine kidney worm can be separated.

Under PL 480 projects, work was reported as follows:

Control of the liver fluke, *Fasciola hepatica* in domestic ruminants.
Fulawy, Poland.

Hexachlorophene was not considered to be satisfactory for mass field use for anti-fluke therapy largely because of its toxic properties. The intramuscular inoculation of carbon tetrachloride, although of high therapeutic value, was unsuitable because of the severe reaction at the point of inoculation.

Studies on the giant liver fluke, *Fasciola gigantica*. Veterinary Faculty,
Ankara University, Ankara, Turkey.

Fasciola gigantica and its intermediate snail host, *Lymnaea auricularia*, were collected in the southern and western parts of Turkey. The snail was also found in the eastern provinces but fascioliasis was not found by examining livers at slaughter houses. If infected ruminants were to be introduced to pastures in this region, this part of the country might also become a problem area. Hexachlorophene destroyed all flukes in sheep and goats when administered 30 to 119 days after experimental exposure. Treatments with carbon tetrachloride did not give consistently good results. Six sheep, exposed to about 100 *Fasciola gigantica* metacercariae, died between the 87th and 113th days after exposure. At necropsy flukes were found in the livers and the livers had hematomas. The prepatent period of *F. gigantica* was 102 to 115 days in sheep, 87 days in goats, and 132 days in one buffalo calf.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

- Becklund, W. W. 1960. Morphological anomalies in male Haemonchus contortus (Rudolphi, 1803), Cobb, 1898 (Nematoda: Trichostrongylidae) from sheep. Proc. Helminth. Soc. Wash., 27:194-199.
- Becklund, W. W. 1961. Helminth infections of healthy Florida cattle, with a note on Cooperia spatulata. Proc. Helm. Soc. Wash., 28:183-84.
- Becklund, W. W. 1961. Helminthiasis of sheep in southern Georgia. Jour. AVMA, 139:781-784.
- Becklund, W. W. 1962. Helminthiasis in Georgia cattle--A clinical and economic study. Amer. J. Vet. Res., 23:510-515.
- Becklund, W. W. 1962. Occurrence of a larval trematode (Diplostomidae) in a larval cestode (Diphyllbothriidae) from Sus scrofa in Florida. J. Parasit., 48:285.
- Chitwood, M. B. 1960. A new spiruroid nematode, Rabbiium caballeroi, from the stomach of Leiocephalus carinatus, from the Bahama Islands. Libro Homenaje al Dr. Eduardo Caballero y Caballero. pp.471-473.
- Diamond, L. S., and F. W. Douvres. 1960. Cultivation of parasitic stages of the swine nematodes Hyostrogylus rubidus and Oesophagostomum quadrispinulatum (O. longicaudum) free of microbial associates. J. Parasit., 46(Suppl.):25.
- Diamond, L. S., and F. W. Douvres. 1962. Bacteria-free cultivation of some parasitic stages of the swine nematodes Hyostrogylus rubidus and Oesophagostomum quadrispinulatum (O. longicaudum). J. Parasit. 48(1):39-42.
- Doss, M. A., J. M. Humphrey, and D. B. Segal. 1961. Index-Catalogue of Medical and Veterinary Zoology, Suppl. 11, Authors: A-Z. 409 pp.
- Doss, M. A., J. M. Humphrey, and D. B. Segal. 1962. Index-Catalogue of Medical and Veterinary Zoology, Suppl. 12, Authors: A-Z. 353 pp.
- Douvres, F. W. 1960. The in vitro cultivation of some gastrointestinal nematodes of cattle and sheep. J. Parasitol. 46(Suppl.):25.
- Douvres, F. W. 1960. Influence of intestinal extracts and sera from cattle infected with Oesophagostomum radiatum on the in vitro cultivation of the nematode: Preliminary report. J. Parasitol. 46(Suppl.):25-26.
- Douvres, F. W. 1962. The in vitro cultivation of Oesophagostomum radiatum, the nodular worm of cattle. I. Development in vitamin-supplemented and non-supplemented media. J. Parasitol. 48(2):314-320.

- Douvres, F. W. 1962. The use of infective larvae and in vitro-grown parasitic larvae of Cooperia punctata to detect antibodies in the serum of a calf infected with this nematode. J. Parasit. 48(Suppl.):15.
- Douvres, F. W., and J. E. Alicata. 1962. Development in vitro of the parasitic stages of Cooperia punctata, an intestinal nematode of cattle. J. Parasit. 48(Suppl.):35.
- Douvres, F. W., and F. G. Tromba. 1962. The development of Stephanurus dentatus Diesing, 1839, to fourth stage in vitro. J. Parasit. 48(2):269.
- Farr, M. M. 1960. Eimeria dunsingi, n. sp. (Protozoa: Eimeriidae) from the intestine of a parakeet, Melopsittacus undulatus (Shaw). Libro Homenaje al Dr. Eduardo Caballero y Caballero. pp.31-35.
- Humphrey, J. M., and D. B. Segal. 1962. A sample of a possible format for annual publication of author, parasite, host and antiparasitic catalogues of the Index-Catalogue of Medical and Veterinary Zoology. J. Parasit. 48, Suppl:49.
- Hwang, J. C., and E. E. Wehr. 1960. Occurrence of Capillaria obsignata Madsen, 1945, in peafowl, with a note on the systematic relationship to some other species of Capillaria in domestic birds. Libro Homenaje al Dr. Eduardo Caballero y Caballero. pp. 475-479.
- Hwang, J. C. 1961. Cladotaenia (Paracladotaenia) cathartidis n. sp. (Destoda: Taeniidae) from the intestine of the turkey buzzard, Cathartes aura septentrionalis Weid, 1893. J. Parasit. 47:205-207.
- McIntosh, A. 1960. A new campulid trematode, Hunterotrema caballeroi n.g., n. sp. from an Amazon dolphin, Inia geoffrensis. Libro Homenaje al Dr. Eduardo Caballero y Caballero. pp. 207-208.
- McIntosh, A. 1962. Book Review--"International code of zoological nomenclature." J. Parasitol. 48:336.
- Tromba, F. G., and L. A. Baisden. 1960. Diagnosis of experimental stephanuriasis in swine by a double diffusion agar precipitin technique. J. Parasitol. 46(Suppl.):29.
- Tromba, F. G. 1962. The immunology of parasitic nematodes. Symp. on the Future of Nematology, 37th Ann. Meet. Amer. Soc. of Parasitologists.

NUTRITION OF ANIMALS AS AFFECTED BY PROPERTIES AND
CHARACTERISTICS OF SOILS AND PLANTS

Soil and Water Conservation Research Division, ARS

Problem. To improve nutrition of animals and man by discovering relationships among soils, plants and animals; to develop basic understanding of the synthesis and metabolism of nutritionally important substances in plants and animals; to determine the functions and pathways of transport of the various elements throughout the food chain from soil to plant to animal; to identify and characterize soil and climatic areas where the nutritional status of animals and man is affected adversely by qualities of the plants produced; to determine the relationships among soil properties, both natural and as modified by treatment, climatic factors, and the nutritional quality of plants as measured chemically or by feeding test animals, so as to enable farmers to select economic combinations of soil, crop and livestock management practices that will meet human nutrition needs.

USDA PROGRAM

The Department has a continuing long-term program involving soil scientists, biochemists and a plant physiologist at the U. S. Plant, Soil and Nutrition Laboratory, a U. S. D. A. laboratory located on the campus of Cornell University. Field and laboratory investigations directed toward understanding of the causes of animal nutrition problems occurring in a geographic pattern suggestive of soil or plant composition vectors are conducted and maps showing the distribution of soil related nutritional problems in livestock are prepared. Plants grown under specific conditions are analyzed for elements and compounds of nutritional significance and at times are fed to test animals to measure their effects on growth, reproduction and metabolic processes. The functions and interrelationships among the micronutrients in the food chain from soil to plant to animal are being determined, and studies of the chemical processes controlling the uptake of micronutrient element by plants are conducted. Work on the synthesis, transamination, and metabolism of amino acids and related substances in plants is underway, with special attention being given the sulfur compounds. An intensive study of the process of protein synthesis is being conducted with the current emphasis placed on the function and structure of the transfer ribonucleic acids.

Studies involving large animals are conducted through contracts with State Agricultural Experiment Stations. During the past year, contract research on molybdenum-copper-sulfur relationships in the nutrition of cattle has been conducted at the University of Nevada. A contract to investigate the effect of soil applications of selenium upon the incidence of myopathy in sheep was initiated at Oregon State University. Work on the causes of congenital malformations in livestock

is conducted in cooperation with the Animal Disease and Parasite Research Division at Logan, Utah.

The Federal scientific effort devoted to research in this area totals 12 professional man-years. Of this number, 1.8 are working to characterize soil and climatic areas where the nutritional status of animals and man is affected adversely by quality of plants produced; 3.4 are devoted to basic understanding of the synthesis and metabolism of nutritionally important substances in plants and animals; 1.8 to biological assays of plant material grown under different soil, geological and other environmental conditions to nutritional disorders in animals and man; and 5.0 to elaboration by plants of vitamins, amino acids, proteins, and other organic nutrient compounds required by animals. The Soil Conservation Service maintains a full time scientist at the Plant, Soil and Nutrition Laboratory for studies relating nutritional problems to specific kinds of soil.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

The State Experiment Stations in 1961 reported a total of 5 professional man-years divided among subheadings as follows: Characterize soil and climatic areas where the nutritional status of animals and man is affected adversely by quality of plants produced, 0.8; basic understanding of the synthesis and metabolism of nutritionally important substances in plants and animals, 2.1; biological assays of plant material grown under different soil, geological and other environmental conditions to nutritional disorders in animals and man, 1.1; and elaboration by plants of vitamins, amino acids, proteins, and other organic nutrient compounds required by animals, 1.0. Soil and climatic areas in which plants cause production problems in livestock are being characterized. The effect of soil fertility upon the synthesis of vitamins, proteins, carbohydrates, and other nutritionally important substances and the effect of soil conditions upon their metabolism in animals is being studied. Biological assay methods are being developed for the rapid determination of nutrient deficiencies or excesses and for identifying the soil, geological and other factors which contribute to the nutritional disorders of man and animals. Attention is being given to the properties of soils and plants which affect the synthesis of vitamins, amino acids, proteins, etc.

There is no research being conducted in this field by industry and other organizations.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Soil and plant composition as factors affecting the distribution of nutritional problems in livestock

Muscular dystrophy in livestock is distributed in a geographic pattern which suggests that soil and plant composition factors may be associated with the prevalence of this nutritional disease. Inasmuch as the addition of very small amounts of selenium to the ration has protected livestock from muscular dystrophy, attention has been focused upon the selenium content of livestock feeds in relation to this problem. Existing evidence does not clearly establish a relationship between the selenium content of the forages from various areas and the incidence of muscular dystrophy in livestock in the area. During the past year, samples of forages have been obtained from a number of farms and ranches where muscular dystrophy was known to be prevalent, and from similar properties where muscular dystrophy is not a problem. These farms and ranches are located in several different parts of the United States. These samples were collected in cooperation with the Animal Husbandry and Veterinary Science Departments of Oregon State University, University of Nevada, Cornell University and the Texas Agricultural Experiment Station. Currently, efforts are being directed toward development of methods for measuring the very small amounts of selenium present in these forage samples, and to comparisons of the selenium content of feeds from farms where muscular dystrophy has been prevalent with those where it has not occurred. The relationships between the content of selenium and other substances which might interfere with selenium metabolism will also be determined.

Along with this survey of the selenium content of forages, contract arrangements have been developed with Oregon State University to study the effect of addition of selenium to the soil upon the incidence of muscular dystrophy in sheep. In this study, selenium will be added to a part of a hayfield on a ranch which has had in the past a high incidence of muscular dystrophy. Hay from the treated portion of the field will be fed to ewes in comparison with hay from the untreated portion, and the incidence of muscular dystrophy in lambs born to these ewes will be determined.

In cooperation with Dr. Wayne Binns of the A. D. P. Division, efforts to clarify the cause of a congenital malformation in lambs have been continued. This malformation results in the birth of lambs with grossly deformed heads and is commonly termed, "monkey face." Since the problem was found only in a few geographic locations, it seemed at first that a mineral deficiency or toxicity in the forage of these areas might be involved. However, during the past two years, Dr. Binns has been able to produce the malformation by feeding the plant, "Veratrum Californicum," to ewes in the early stages of gestation.

The past year's work has, therefore, centered around determination of the specific components in Veratrum which are responsible for the malformation, and toward explanation of why Veratrum collected from different sites varies in its effect in producing the malformation.

In order to efficiently pursue this research, an attempt has been made to use the rat as a laboratory animal for assay of the toxic principle. A partial fractionation, designed to separate the alkaloids, was performed on a sample of Veratrum from a collection which had previously caused the malformation in sheep. The various fractions were fed to rats during the gestation period. In addition, several samples of the whole plant, from collections of varying toxicity to sheep, were fed to the rats under similar conditions. In this experiment, no interferences with gestation and the production of normal litters of rats was observed. This is in contrast to previous results at this laboratory using a similar assay technique. In a second experiment, it was found that a sample of Veratrum which, in 1959, had markedly interfered with gestation in the rats was no longer toxic at the levels previously fed (3% of the diet), but that a sample of more recent collection known to cause the malformation in sheep would interfere with gestation provided it was fed at a high level (10% of the diet). These results indicate that the toxic principle in Veratrum may be labile and decompose in stored samples, and that the level of the toxic principle in the plant may vary from season to season. Research designed to identify the toxic principle and account for its variations in the plants will be continued.

An intensive survey of the cobalt and copper concentrations in forages in New England was conducted in cooperation with the Soil Conservation Service. The objective of this study is to accurately define the soil areas in New England which produce forages containing insufficient cobalt to meet the requirements of livestock. Nearly 500 forage samples from about 250 different soil sites were collected and are currently being analyzed. From preliminary results, it appears that the major cobalt-deficient areas in New England will be located on Podzol soils developed on granitic glacial drift originating in the White Mountains of New Hampshire.

The molybdenum content of vegetation growing on poorly drained wet soils is often higher than that found in similar plants growing on the better drained and drier soils formed from the same parent material. A study of the effect of soil moisture level per se upon the uptake of micronutrients by plants might serve to explain variations in micronutrient contents of plants growing on different kinds of soils and in different seasons. For this reason, a greenhouse study has been conducted to measure the influence of soil moisture level upon the uptake of trace elements by alsike clover. Four soils with a known history of cobalt deficiency or molybdenum toxicity are included in the experiment. Alsike clover is grown at a high moisture level (water table at

7" depth) and a low moisture level (0.3 to 10 atmospheres suction) on each soil.

In the first two cuttings from this experiment, the molybdenum content of the plants was about twice as great on the wet treatments as on the dry treatments on all soils. These findings indicate that a high moisture level per se is a factor in the high molybdenum content found in vegetation growing on wet soils, and that improved drainage of these soils may help to reduce the molybdenum content of the forage.

In cooperation with the University of Georgia, Animal Husbandry Department, and the Southern Piedmont Conservation Experiment Station, a study of the effect of nitrogen fertilization upon the nutritional quality of Coastal Bermuda hay was conducted. Two lots of hay, one produced with an annual application of 100 lbs. N per acre, and one produced with an annual application of 675 lbs. N per acre, were fed to grade Angus steer calves for 140 days in a ration of hay, salt and cottonseed meal. The steers fed the hay produced with the high level of nitrogen gained 1.11 lbs. per day during this period, and consumed 12.5 lbs. of feed per pound of gain. The steers fed the hay produced at the low level of nitrogen fertilization gained .96 pound per day and consumed 14.1 lbs. of feed per pound of gain. Thus, under the conditions of this experiment, the hay produced at the high level of nitrogen fertilization was slightly superior to the hay produced at the low level of N fertilization.

Due to the recent findings that nitrates in feeds may interfere with the conversion of carotene to vitamin A in the animal, nitrates were determined on the hays and levels of carotene and vitamin A were determined on the blood of the experimental animals. The hay produced at the high nitrogen level contained an average of 1,100 parts per million of nitrate nitrogen, and that produced at the low level of nitrogen fertilization contained an average of 219 parts per million of nitrate nitrogen. The blood carotene tended to be higher and the blood vitamin A lower in the steers fed the high nitrogen hay than in the steers fed the low nitrogen hay. However, even in the steers fed the high nitrogen hay, the levels of vitamin A in the blood were above generally recognized critical minimums.

Following the termination of the experiment comparing the nutritional quality of Coastal Bermuda hay as affected by level of nitrogen fertilization, the steers used were pastured as one group for the summer at the Southern Piedmont Conservation Experiment Station. During this summer pasture period, these steers gained very little weight, even though other animals on similar pastures at the same station gained satisfactorily. The cause of the low summer gains on the experimental steers will be studied in future work on this project.

Many relationships of soils and soil management to the nutritional quality of plants are concerned with forage crops. Bioassay procedures, utilizing laboratory animals, are needed for the efficient study of some of these problems. Inasmuch as the rabbit will utilize diets containing a high percentage of forages, efforts have been directed toward the development of diets for rabbits to use as a base in the nutritional evaluation of forages.

The basal diet employed in these studies contained glucose, cellulose, corn oil, a purified protein, minerals, and vitamins. Several of the mineral requirements of the rabbit have been determined with this diet.

The results indicate that the rabbit requires 0.80% potassium, 0.30% sodium and 0.15% magnesium to achieve maximum performance. Attempts to produce a zinc deficiency have been unsuccessful. Therefore, it is concluded that the zinc requirement of the growing rabbit does not exceed the zinc supplied by the basal diet which contains 5-7 parts per million zinc.

B. Trace element interactions and functions in animal nutrition

Studies of the factors affecting the copper nutrition of animals--especially the copper-molybdenum-sulfate interrelationships--are continuing at this laboratory in cooperation with the Biochemistry Department of Cornell University. These studies are important for a better understanding of field problems involving the copper nutrition of grazing animals. Many of the observations with respect to Cu-Mo interrelationships and the effect of molybdenum on the copper status of the animal are conflicting. A level of molybdenum which has been found to be harmless under one set of conditions has produced toxic symptoms under another; or, a level of copper may be adequate at one location and the same level will not prevent a frank copper deficiency at another.

Experiments conducted several years ago at this laboratory demonstrated a beneficial effect of methionine on molybdenum toxicity in the animal. Studies in other laboratories confirmed this finding and added the information that inorganic sulfate was equally beneficial.

At about the same time that these observations were made using the rat as the experimental animal, studies by others with sheep showed that dietary sulfate accentuated a molybdenum-induced copper deficiency.

These completely contradictory findings with respect to the beneficial effect of sulfate in one instance and the detrimental effects in another were most puzzling. It was postulated that the apparent contradiction might be due to species difference--the ruminant vs. the monogastric animal.

Recent work at the laboratory has shown that the rat behaves like the sheep under certain conditions. The early work was planned to study molybdenum toxicity per se. Copper was included in all diets to meet adequately the copper requirement of the rat. The recent experiments were planned to study the effect of dietary molybdenum on copper-depleted animals, a condition more often encountered with field problems in which copper is a factor. A depletion period of about two weeks reduced the copper and iron stores of the weanling rats to a point of relatively severe anemia. When a low level of molybdenum (.001% Mo) was then incorporated into (-Cu) diets, a significant growth retardation occurred; when sulfate was also included with the molybdenum, the deleterious effect of this level of molybdenum was greatly accentuated. The animals fed this level of molybdenum gained approximately 2/3 as much as the animals on the minus Cu basal diet, while those fed Mo + SO₄ lost weight during the experimental period.

At the same time that these Cu-depleted animals were on experiment, another group of weanling rats (from the same breeding of the laboratory stock colony) was placed on Cu-adequate diets. Thus, these animals from a single breeding were studied under identical laboratory conditions, the only difference between the (+Cu) and the (-Cu) animals being their copper status. As in the earlier work with Cu-adequate diets, a higher level of dietary molybdenum (.08% Mo) resulted in about a fifty percent growth retardation. When sulfate was incorporated with molybdenum in the diets, the toxic effect of the molybdenum was eliminated. Sulfate was added at the same level to both the Cu-deficient and the Cu-adequate diets.

The results clearly demonstrated that sulfate fed with a toxic level of molybdenum to animals with a Cu-adequate status corrected the growth retarding effects of the molybdenum; and contrariwise, sulfate added to diets of Cu-depleted animals accentuated the toxic effects of dietary molybdenum. Eighty times as much molybdenum was needed to produce molybdenum toxicity in the instance of the Cu-adequate animals compared with that used with the Cu-deficient animals. While methionine exerted the same alleviating effects on molybdenum toxicity as in the earlier experiments (approximately 60% correction), Na₂SO₄ (sulfur equivalent to the methionine sulfur) completely corrected the toxic effects of molybdenum fed to Cu-adequate animals. The Mo + Cu + SO₄ treatment was no better than the Mo + SO₄ treatment-- a further indication of the Cu-adequate status of these animals.

In the experiments with sheep, it was shown that sulfate must be present for molybdenum to exert an influence on the reduction of copper stores in the liver. A study is planned of the copper and molybdenum levels in the livers of rats (Cu-deficient and Cu-adequate), as affected by dietary sulfate.

Through contract arrangements with the University of Nevada, interactions of molybdenum with other elements have also been studied using beef cattle. The major experiment conducted during the past year was designed to measure the effect of level of protein and level of sulfate upon the toxicity of molybdenum. The basal ration was grass hay. Molybdenum (100 parts per million as inorganic molybdate), sulfate (.5% as sodium sulfate) and supplemental protein (6 lbs. alfalfa per day) were added to the basal diet, singly and in all combinations. Yearling heifers were maintained on these rations for ten months. Using weight gains as a criteria, the addition of sulfate to high molybdenum rations did not increase the toxic effects of the molybdenum.

This effect of sulfate is in contrast to that found in the experiment with copper-deficient rats described in this report, and is also in contrast to some of the results obtained by Australian workers. Biochemical measurements of the copper and sulfur status of the cattle will be conducted utilizing tissues collected during the feeding trial, in order to establish the reasons for this contrast.

In an auxiliary experiment at Nevada, animals grazing a normal low molybdenum irrigated pasture were treated daily with amounts of molybdenum estimated to be equivalent to 50 and 100 parts per million of molybdenum in the dry matter intake. The molybdenum was added as inorganic molybdate in gelatin capsules. The differences between control animals and molybdenum treated animals was much more pronounced under grazing conditions than in the dry lots. This indicates an increased toxicity of the same amount and chemical form of molybdenum to animals on fresh green forage as contrasted to animals on dry hay. This finding substantiates the belief of some ranchers that certain fields may be safely used as hay fields, but will result in molybdenosis when used for pasture.

During the past year, studies of the function of trace elements in the formation of bone have centered around the role of manganese in the formation of the organic matrix. Previous work at this laboratory has indicated that manganese deficiency in chicks results in a reduction in the mucopolysaccharide content of the epiphyseal cartilage of certain bones. The mucopolysaccharides that contain galactosamine are the most markedly reduced in these cartilages.

These findings offer a possible biochemical explanation for the bone abnormalities which occur in manganese-deficient chicks. Recent experiments have been concerned with the specificity of the manganese effect upon cartilage mucopolysaccharides. It has been established that a reduced food intake will not give rise to the reduction in mucopolysaccharides observed in manganese-deficient chicks. Experiments with graded levels of dietary manganese demonstrated a close relationship between manganese intake and the mucopolysaccharide content of the epiphyseal cartilage.

In chicks suffering from rickets (Ca deficiency) an increased mucopolysaccharide content is observed even at a low level of manganese intake. Increasing the manganese level to adequate amounts will bring about further increases in mucopolysaccharides in the cartilages. These studies will be continued looking toward clarification of the biochemical functions of manganese in bone formation.

The trace element content of a number of tissues from sheep is being determined in support of the research of Dr. Binns of the A.D.P. Division. The objective of this research is to determine whether or not various trace elements are accumulated in specific tissues, as an aid to the diagnosis of problems of mineral deficiency and toxicity.

C. Biosynthesis of amino acids, peptides and proteins

Studies of the intermediate chemical processes in the biosynthesis of proteins have been continued in cooperation with the Department of Biochemistry of Cornell University. The work has been centered on investigations of the nature of the soluble ribonucleic acids (RNAs) that transfer activated free amino acids to the "template" where protein synthesis takes place. A major objective of this work was attained during the year with the separation of three of the twenty amino acid specific yeast ribonucleic acids in form sufficiently pure for studies of their structure, and free of activity for the transfer of any other amino acid. The purified RNAs obtained are those which transfer alanine, tyrosine and valine. Structural studies have been initiated on these three ribonucleic acids. They are known to consist of long chains of four or five different nucleotide units and the structure problem is initially one of determining the number and sequence of units along the chain. The chains can be broken down to the individual nucleotide units by hydrolysis with KOH. Analyses of the nucleotides obtained in this way indicate that the alanine ribonucleic acid, as compared with the other two, has a very low content of adenylic acid and a high content of guanylic acid. The valine and tyrosine ribonucleic acids differ little in nucleotide composition. The RNA chains may be broken down to somewhat larger fragments called oligonucleotides by the action of the enzyme pancreatic ribonuclease. Identification of the oligonucleotides reveals the sequence of the individual nucleotides in short fractions of the RNA chain. Analyses of the compositions of pancreatic ribonuclease digests of the three ribonucleic acids, using a newly developed method of chromatography on DEAE-Sephadex, has established that they differ greatly in nucleotide sequence. Hardly a single oligonucleotide fragment is found to the same extent in pancreatic ribonuclease digests of the three RNAs. It is concluded that the three ribonucleic acids differ greatly in structure.

A simple method for the preparation of the amino acid-specific ribonucleic acids from mammalian liver was developed. This procedure, when used with beef or hog liver, should make the mammalian ribonucleic acids readily available and will facilitate studies of species differences in the ribonucleic acids.

During recent years, this laboratory has been successful in isolating and identifying a series of heretofore little known nitrogen compounds from plants. Several of these compounds are dipeptides in which the gamma carboxyl group of glutamic acid is involved in the peptide linkage. In some plant tissues, these γ -glutamyl dipeptides contain an important part of the total plant nitrogen. For this reason, knowledge of the identity of these compounds, their mode of formation, and their function in the plant needs to be explored in order to establish their significance in nitrogen metabolism, nutrition, and their relationship to toxic substances.

During the past year, three additional γ -glutamyl dipeptides have been isolated in crystalline form and their identity conclusively proven by comparison with synthetic dipeptides. One of these dipeptides (γ -glutamyl β -alanine) was isolated from iris bulbs. This peptide is very similar structurally to the "lathyrus factor" - the toxic principal of sweet peas. Possibly they are interconverted in the plant. γ -Glutamyl β -alanine has not previously been reported to occur naturally. β -Alanine was also isolated out of this tissue and compared with authentic material. Although β -alanine is widely reported in plants, this is the first time it has been isolated from a higher plant.

Soybean seeds were the source of the two other isolated dipeptides. These compounds have been shown to be γ -L-glutamyl-L-tyrosine and γ -L-glutamyl-L-phenylalanine. The identity was conclusively proven by comparison with synthesized dipeptides. The tyrosine dipeptide has not previously been reported to exist in plants although the phenylalanine peptide has been isolated from onion bulbs.

It is noteworthy that there was about 500 times as much β -alanine in the dipeptide form than unbound in the iris tissue. An analogous situation is found in soybeans where there was twenty-seven times as much tyrosine and seven times as much phenylalanine in the dipeptide form as in the free form.

In addition, two other γ -glutamyl dipeptides have been isolated from kidney bean seeds. These are probably γ -glutamyl leucine and γ -glutamyl methionine.

In an effort to learn something about the biosynthesis of the γ -glutamyl dipeptides, a search was made for an enzyme called glutathionase or transpeptidase. This enzyme is known to occur in animals and is presumably responsible for the degradation of glutathione. The enzyme transfers the γ -glutamyl group of glutathione to an amino acid to produce a dipeptide. Although transpeptidase had not been shown to occur in plants, it was readily demonstrated in a number of legume tissues. The enzyme was purified from kidney bean fruits and extensively studied. It was clearly demonstrated that the enzyme synthesizes γ -glutamyl dipeptides. It is probably responsible for the biosynthesis of the γ -glutamyl peptides of methylcysteine, methionine, leucine, phenylalanine and tyrosine. The enzyme does not form γ -glutamyl β -amino-isobutyric acid nor γ -glutamyl β -alanine. The pathway of formation of the latter two dipeptides is not known. The plant transpeptidase is quite different from the animal enzyme with respect to (1) location within the cell, (2) specificity towards amino acid acceptors, (3) inhibition by various sulfonphthaleins, and (4) activation by magnesium ions.

The work on the biosynthesis of dipeptides was particularly aimed at the formation of γ -glutamyl-methylcysteine in kidney bean seeds where it comprises nearly half of the non-protein nitrogen. Methylcysteine is of considerable interest because it is a sulfur containing amino acid. The essential sulfur containing amino acids are frequently the limiting amino acids in the utilization of plant proteins by humans and animals.

Although the sulfoxide of methylcysteine has been known for several years to be a prominent constituent of plants of the mustard family, methylcysteine has not been reported in these plants. Indirect evidence was obtained that methylcysteine also is present in these species. Intact crucifer tissues rapidly oxidize methylcysteine to its sulfoxide. This process is apparently enzymatic since heating at 100° for one minute abolishes the oxidation. The enzyme system is quite labile as evidenced by its inactivation by freezing, chloroform treatment, and mechanical disruption of tissues. The lability of the enzyme has prevented the preparation of a cell-free system which will oxidize methylcysteine.

In order to facilitate studies of nitrogen and sulfur metabolism in green plants, attempts have been made to induce biochemical mutants in chlorella by ultra-violet radiation. One mutant which could not utilize nitrate, but could use nitrate or ammonia, has been shown to lack nitrate reductase.

The amino acid distribution in oat forage as affected by nitrogen and sulfur supply was determined in support of the research on sulfur fertilization conducted by the Southeast Branch, SWC Division. The content of sulfur amino acids in oat forage was, in general, proportional to the total protein content.

D. Chemistry of micronutrients in soils

Studies conducted in cooperation with the Soil Conservation Service have served to establish the effects of weathering and soil genesis upon the distribution and availability of cobalt in the soils of Eastern United States. Two major processes tend to control cobalt distribution in soil profiles. The first process is downward movement of cobalt in the soil profiles through the leaching action of downward moving water. This downward movement is evidenced by the fact that in weathered soils the maximum concentration of total cobalt in the soil is generally in the B horizon.

The second process involved is an upward movement of cobalt, due to the fact that plants take up cobalt from various depths in the profile and deposit it on the surface with the plant residues. The existence of this process is revealed when samples of soil from various depths in the profile are extracted with dithionite or with 2.5% acetic acid, and the amount of cobalt extracted is determined.

The cobalt extracted by dithionite or 2.5% acetic acid is the most soluble and chemically reactive fraction of the total soil cobalt. This fraction can be considered to represent the circulating or "free" part of the cobalt. Where the maximum concentration of circulating cobalt is found at a higher level in the soil profile than the maximum concentration of total cobalt, the process of upward movement through the plant is keeping pace with downward movement through the soil, and the supply of available cobalt in surface horizons is being maintained. Where the maximum concentration of circulating cobalt is found deep in the profile, below the maximum of total cobalt, downward movement and loss from the soil is the dominant process, and ultimate development of a cobalt-deficient soil will result.

Measurements of the cobalt content of plants are in accord with this theory. In Gray-Brown and Red-Yellow Podzolic soils, the maximum concentration of dithionite or acetic acid soluble cobalt is found in the surface horizons, either the A₁ or the A₂. These soils almost always support vegetation containing adequate amounts of cobalt for grazing animals. In Podzols and Humus Ground Water Podzol soils, the maximum concentration of dithionite extractable cobalt is found below the maximum concentration of total cobalt. The cobalt content of the vegetation on these soils is frequently less than is needed to meet the requirements of grazing animals.

Investigations of the nature of the reaction between soluble cobalt and the solid particles in soils have been continued. An understanding of these reactions is required in order to develop efficient procedures for using cobaltized fertilizers to correct cobalt deficiencies in grazing animals. The work of the past year has been focused upon role of clay minerals, organic matter, and iron

coatings in the processes through which soluble cobalt is immobilized in the soil.

It was concluded from previous work that the sites of the reaction of cobalt with silicate clays are concentrated in zones around defects in the crystalline structure of the clay. These defects in crystalline structure apparently can result from weathering of the mineral surfaces or from laboratory treatments of the clay. The weathered nature of the soil surfaces is likely to have a greater influence on the reaction of a soil with soluble cobalt than does the average mineralogical composition of the soil. The iron coatings on the surfaces of the soil particles are involved in the reaction of these particles with cobalt. This is shown by the fact that removal of the iron coatings from a group of soils brought about changes in the reactivity of some of these soils with cobalt. A search for a method for removing heavy metal-organic matter complexes from soil has not, up to the present, been successful. Techniques based on destruction of the organic fraction with hydrogen peroxide have been found to dissolve cobalt from sources other than organically bound forms in the soil.

As another part of the continuing research program on the chemistry of trace elements in soils at this laboratory, an extensive review of the literature in this area is underway. This review covers copper, cobalt, zinc, iron, manganese, molybdenum and boron. A card system that will permit rapid survey of existing research in this field is being designed.

A requirement for cobalt by legumes in order to fix nitrogen has been established at other laboratories. An experiment has been conducted to determine whether or not some of the soils which produce vegetation with a very low concentration of cobalt can supply legumes with sufficient cobalt for normal plant growth. No definite responses to cobalt could be observed in alfalfa growing on any of the soils in the light chamber. The content of cobalt in the plant was very near the value found by other workers in soybeans suffering from insufficient cobalt. It would, therefore, appear that the level of available cobalt in these soils is marginal for legumes. Responses to cobalt on these soils might be found in the field under favorable growing conditions, but it is not likely that they would be large. It also appears that cobalt deficiency in grazing animals will be encountered before plant growth is restricted from lack of available cobalt.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Soil and Plant Composition as Factors Affecting the Distribution of
Nutritional Problems in Livestock

- Allaway, W. H., and Beeson, K. C. 1960. Fertilizers and lime in relation to the nutritive value of crops. Internatl. Soc. Soil Sci. 7th Cong. Trans., 3(4), pp. 12-19.
- Kubota, Joe, Lazar, Victor A., and Beeson, Kenneth C. 1960. The study of cobalt status of soils in Arkansas and Louisiana, using the black gum as the indicator plant. Soil Sci. Soc. Amer. Proc., 24, pp. 527-528.
- Allaway, W. H. 1961. Animal health. Crops and Soils, 13, p. 7.
- Allaway, W. H. 1961. Soils, fertilizers, and crop quality. Proc. 12th Annual Fertilizer Conf. of the Pacific Northwest, pp. 1-8.
- Allaway, W. H. 1961. Trace elements in the soil and the nutritional quality of crops. Crops and Soils, 13(7), p. 10.
- Kubota, Joe, and Lazar, Victor A. 1961. Cobalt-iron relationship in sandy soils. Internatl. Soc. Soil Sci. 7th Cong. Trans., 2(2), pp. 134-141. (1960)
- Kubota, Joe, Lazar, Victor A., Langan, L. N., and Beeson, Kenneth C. 1961. The relationship of soils to molybdenum toxicity in cattle in Nevada. Soil Sci. Soc. Amer. Proc., 25, pp. 227-232.
- U. S. Plant, Soil and Nutrition Laboratory Staff. 1961. Cobalt deficiency in soils and forages: How it affects cattle and sheep. U.S.D.A. Leaflet 488.

Trace Element Interactions and Functions in Animal Nutrition

- Leach, R. M., Jr. 1961. Mineral interrelationships in poultry nutrition. Cornell Nutr. Conf. for Feed Mfgs., pp. 38-41.

Biosynthesis of Amino Acids, Peptides and Proteins

- Everett, George A., Merrill, Susan H., and Holley, Robert W. 1960. Separation of amino acid-specific "soluble" ribonucleic acids by partition chromatography. Amer. Chem. Soc. Jour., 82, pp. 5757-5758.
- Apgar, Jean, Merrill, Susan H., and Holley, Robert W. 1961. Counter-current distribution of yeast "soluble" ribonucleic acids in a modification of the Kirby system. Biochem. Biophys. Acta, 53, pp. 220-221.
- Baker, James E., and Thompson, John F. 1961. Assimilation of ammonia by nitrogen-starved cells of Chlorella vulgaris. Plant Physiol., 36(2), pp. 208-212.
- Doctor, B. P., Apgar, Jean, and Holley, Robert W. 1961. Fractionation of yeast amino acid-acceptor ribonucleic acids by countercurrent distribution. Jour. of Biol. Chem., 236(4), pp. 1117-1120.
- Everett, George A., and Holley, Robert W. 1961. Effect of minerals on amino acid incorporation by a rat-liver preparation. Biochem. Biophys. Acta, 46, pp. 390-391.

- Holley, Robert W., Apgar, Jean, Doctor, B. P., and Others. 1961. A simplified procedure for the preparation of tyrosine- and valine-acceptor fraction of yeast "soluble-ribonucleic acid." Jour. Biol. Chem., 236, pp. 200-202.
- Holley, Robert W., Apgar, Jean, and Merrill, Susan H. 1961. Evidence for the liberation of a nuclease from human fingers. Jour. Biol. Chem., 236 (7), p. 42.
- Holley, Robert W., Apgar, Jean, Merrill, Susan H., and Zubkoff, Paul L. 1961. Nucleotide and oligonucleotide compositions of the alanine-, valine-, and tyrosine-acceptor "soluble" ribonucleic acids of yeast. Jour. of Amer. Chem. Soc., 83, p. 4861.
- Holley, Robert W., Brunngraber, Elinor F., Saad, F., and Williams, Harold H. 1961. Partial purification of the threonine- and tyrosine-activating enzymes from rat liver, and the effect of potassium ions on the activity of the tyrosine enzyme. Jour. Biol. Chem., 236, pp. 197-199.
- Holley, Robert W., and Lazar, Victor A. 1961. Metal content of "soluble" fraction ribonucleic acids. Jour. Biol. Chem., 236(5), pp. 1446-1447.
- Honda, S. I., Baker, J. E., and Muenster, Anna-Marie. 1961. An f-type cytochrome from Chlorella vulgaris. Plant and Cell Physiol., 2, pp. 151-163.
- Honda, S. I., and Muenster, Anna-Marie. 1961. Some effects of toxicity on lupine succinoxidase. Plant Physiol., 36, pp. 105-112.
- Morris, Clayton J., Thompson, John F., Asen, Sam., and Irreverre, Filadelfo. 1961. Isolation of beta-alanine from Iris Tingitana (var. Wedgewood). Nature, 190, 4777, pp. 718-719.
- Morris, Clayton J., Thompson, John F., Asen, Sam., and Irreverre, Filadelfo. 1961. The isolation of γ -L-glutamyl- β -aminoisobutyric acid from iris bulbs. J. Biol. Chem., 236(4), pp. 1181-1182.
- Shafer, John, Jr., Baker, James E., and Thompson, John F. 1961. A chlorella mutant lacking nitrate reductase. Amer. Jour. of Botany, 48(10), pp. 896-899.
- Thompson, John F., Morris, Clayton J., Asen, Sam., and Irreverre, Filadelfo. 1961. m-Carboxyphenyl-L-alanine: A dicarboxylic aromatic amino acid from iris bulbs. J. Biol. Chem., 236(4), pp. 1183-1185.
- Apgar, Jean, Holley, Robert W., and Merrill, Susan H. 1962. Purification of the alanine-, valine-, histidine-, and tyrosine-acceptor ribonucleic acids from yeast. Jour. of Biol. Chem., 237(3), pp. 796-802.
- Arnold, Wilfred Niels, and Thompson, John F. 1962. The formation of (+) S-Methyl-L-Cysteine Sulfoxide from S-Methyl-L-Cysteine in Crucifers. Biochem. Biophys. Acta 57, pp. 604-606.

Chemistry of Micronutrients in Soils

- Kabata, Alina, and Beeson, Kenneth C. 1961. Cobalt uptake by plants from cobalt impregnated soil minerals. Soil Sci. Soc. Amer. Proc., 25(2), pp. 125-128.
- Kubota, Joe., and Lazar, Victor A. 1961. Cobalt-iron relationship in sandy soils. Internatl. Soc. Soil Sci. 7th Cong. Trans., 2(2), pp. 134-141.

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POULTRY RESEARCH

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of the
United States Department of Agriculture
and related work of the
State Agricultural Experiment Stations

This progress report is primarily a research tool for use of scientists and administrators in program coordination, development, and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of research progress include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members, and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued during the past year. Current agricultural research findings are also published in the monthly U.S.D.A. publications, Agricultural Research and The Farm Index.

UNITED STATES DEPARTMENT OF AGRICULTURE
Washington, D. C. 20250

December 1, 1964



ADVISORY COMMITTEES

The research program of the Department of Agriculture is reviewed annually by the following advisory committees:

1. Farm Resources and Facilities Research
2. Utilization Research and Development
3. Human Nutrition and Consumer Use Research
4. Marketing Research
5. Agricultural Economics Research
6. Forestry Research
7. Animal and Animal Products Research
8. Cotton Research
9. Grain and Forage Crops Research
10. Horticultural Crops Research
11. Oilseed, Peanut and Sugar Crops Research
12. Plant Science and Entomology
13. Tobacco Research

ORGANIZATIONAL UNIT PROGRESS REPORTS

The source materials used by the advisory committees are of two types. First, there are Organizational Unit Reports that cover the work of the Divisions or Services listed below. The number prefixes refer to advisory committees listed above that review all of the work of the respective Divisions or Services.

Agricultural Research Service (ARS)

- 1 - Agricultural Engineering
- 1 - Soil & Water Conservation
- 2 - Utilization -- Eastern
- 2 - Utilization -- Northern
- 2 - Utilization -- Southern
- 2 - Utilization -- Western
- 3 - Human Nutrition
- 3 - Clothing and Housing
- 3 - Consumer & Food Economics
- 4 - Market Quality
- 4 - Transportation & Facilities
- 7 - Animal Husbandry
- 7 - Animal Disease & Parasite
- 12 - Crops
- 12 - Entomology

Economic Research Service, (ERS)

- 4,5 - Marketing Economics
- 4 - Farm Production Economics
- 5 - Resource Development Economics
- 5 - Economic & Statistical Analysis
- 5 - Foreign Development & Trade Analysis
- 5 - Foreign Analysis

Other Services

- 4,5 - Farmer Cooperative Service (FCS)
- 4,5 - Statistical Reporting Service (SRS)
- 6 - Forest Service (FS)

SUBJECT MATTER PROGRESS REPORTS

The other type of report brings together the U.S.D.A. program and progress for the following commodities or subjects:

- | | |
|--|------------------------------------|
| 3 - Rural Dwellings | 8 - Cotton and Cottonseed |
| 6 - Forestry (other than Forest Service) | 9 - Grain and Forage Crops |
| 7 - Beef Cattle | 10 - Citrus and Subtropical Fruit |
| 7 - Dairy | 10 - Deciduous Fruit and Tree Nut |
| 7 - Poultry | 10 - Potato |
| 7 - Sheep and Wool | 10 - Vegetable |
| 7 - Swine | 10 - Florist, Nursery & Shade Tree |
| 7 - Cross Specie and Miscellaneous | 11 - Oilseeds and Peanut |
| Animal Research | 11 - Sugar |
| | 13 - Tobacco |

A copy of any of the reports may be requested from Max Hinds, Executive Secretary, Animal and Animal Products Research Advisory Committee, Research Program Development and Evaluation Staff, U. S. Department of Agriculture, Washington, D. C.

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INTRODUCTION

This report on poultry research covers work directly related to the production, processing, distribution, and consumption of poultry and poultry products. The information has been assembled from the organizational unit reports of the several divisions. This report does not include extensive cross-commodity work, much of which is basic in character, which contributes to the solution of not only poultry problems but also to the problems of other commodities. Progress on cross-commodity work is found in the reports of the several divisions such as Soil and Water Conservation, Human Nutrition, Transportation and Facilities, Farm Production Economics, Foreign Development and Trade Analysis, and Cross-Species and Miscellaneous Animal Research.

This report is devoted to the 22 "problem areas" shown in the table of contents. For each area there is a statement of (1) the Problem, (2) the USDA and Cooperative Program, (3) Program of State Experiment Stations, (4) a summary of Progress during the past year on USDA and cooperative work, and (5) a list of Publications resulting from USDA and cooperative work.

Research on poultry and poultry products can be divided into three major categories, i.e., that supported by (1) Federal funds appropriated to the research agencies of the United States Department of Agriculture, (2) Federal and State funds appropriated to the 53 State Agricultural Experiment Stations, and (3) private funds allotted, largely by poultry industries, to research carried on in private laboratories or to support of State Station or USDA work. For all three categories it is estimated that about 1,100 scientists are engaged in research dealing specifically with the production, processing, distribution, and consumption of poultry and poultry products. Support of their work involves an annual expenditure of between 25 and 30 million dollars. This amounts to about 0.8 percent of the cash farm receipts from poultry and eggs and about 0.5 percent of the retail cost of poultry and eggs. Of the 1,100 scientists engaged in poultry research, approximately 13% are employed by the Department of Agriculture, 27% by the State Experiment Stations, and 60% by other universities, foundations, and private industry.

Research by USDA

Farm research pertaining to poultry is conducted in the Agricultural Research Service divisions of Agricultural Engineering, Animal Disease and Parasite, Animal Husbandry, and Entomology. The work comprises investigations of breeding, physiology, nutrition, viability, diseases, insects, housing and management, involving 86 professional man-years of scientific effort.

Nutrition, consumer, and utilization research pertaining to poultry is conducted in the Agricultural Research Service divisions of Human Nutrition, Consumer and Food Economics, and Western Utilization. The work comprises investigations of composition and nutritive value; physiological availability of nutrients and their effects; new and improved methods of preparation, preservation, and care in homes, eating establishments and institutions; and with the processing phase involving slaughtering the birds and processing the meat and eggs. Also, it is concerned with improved equipment and processes. The work in these divisions involves 35 professional man-years of scientific effort.

Marketing and economic research pertaining to poultry is carried on within four Services: Agricultural Research Service, Economic Research Service, Farmer Cooperative Service, and Statistical Reporting Service. The work comprises (1) physical and biological aspects of assembly, packaging, transporting, storing and distribution; (2) economic aspects of marketing costs, margins and efficiency, market potential, supply and demand, and situation and outlook; (3) cooperative marketing, and (4) consumer acceptance studies. The divisions in which the work is conducted are: Market Quality, ARS; Transportation and Facilities, ARS; Marketing Economics, ERS; Economic and Statistical Analysis, ERS; Marketing Division, FCS; Standards and Research, SRS. The scientific effort involved by these divisions amounts to 32 professional man-years.

Interrelationships among Department, State and Private Research

A large part of the Department's research is cooperative with State Experiment Stations. Many Department employees are located at State Stations and use laboratory and office space close to or furnished by the Station. Cooperative work is jointly planned, frequently with the participation of representatives of the producers or industry affected. The nature of cooperation varies with each study. It is developed so as to fully utilize the personnel and other resources of the cooperators which frequently includes resources contributed by the interested producers or industry.

Including both cooperative and State Station projects poultry research is carried on by 51 State Experiment Stations. The types of work to which the largest amount of effort is devoted include nutrition, physiology and management, breeding, diseases and economics of marketing. There is regular exchange of information between Station and Department scientists to assure that the programs complement each other and to eliminate unnecessary duplication.

Privately supported poultry research emphasizes the solution of scientific production, processing, and marketing problems. Much of it utilizes the results of more basic work done by State Station and Department scientists.

Major areas of emphasis include poultry nutrition and breeding. This is peculiar to the poultry industry. For other animal species much of the nutrition and breeding work is done with public research. Private research in processing is devoted largely to control methods, standardization of products, and product quality and formulation. Research in marketing and economics by industry is in connection with new product development and in merchandising and promoting farm products. Industry participates heavily in consumer acceptance research but largely with respect to a firm's own brand name.

The contributions of poultry producers and industry to the work of the State Stations and the Department have been an important factor in the success of their research programs. Producers offer flocks and facilities for testing products and practices used in production. Likewise, processors and retailers offer facilities and products for use by public research agencies. Many problems in the economics of marketing cannot be transferred to a laboratory, experimental plot, or other simulated situation. The results of economic research conducted cooperatively is of great value to industry, especially in cases where public research can provide comparison and analysis. Even large firms that have a research staff do not have access to the plants and records of competitors.

Examples of Recent Research Accomplishments by USDA and Cooperating Scientists

Removal of the bursa gland reduces leukosis in chickens. Research at the Regional Poultry Research Laboratory at East Lansing, Michigan, and cooperators indicates that the surgical removal of the bursa gland (bursa of Fabricius), in most cases, prevented them from contracting visceral lymphomatosis. This was also the case when both the bursa and thymus gland were removed but removal of only the thymus gland had no detectable effect on the incidence of the disease. Surgery was performed on young chickens to remove the bursa, the thymus, or both. Neither gland was removed from a group of chicks maintained as controls. All were inoculated with a cultured RPL 12 leukosis virus, some before surgery and some after. The growth and development was not affected by the surgery. Bursa removal was effective whether the operation was at 2 or 29 days of age and whether the chickens were infected at 1 day or 28 days of age. Even when the virus was administered to day-old chicks and the operation was performed 4 weeks later, bursectomy drastically reduced the incidence of visceral lymphomatosis. This is particularly significant because the RPL virus multiplies in chickens to such an extent that much virus can be found in saliva and droppings by 4 weeks of age. Of considerable interest was the failure of either or both thymectomy and bursectomy to influence the incidence of other forms of leukosis--erythroblastosis or osteopetrosis. Previous research has shown that RPL 12 virus takes different forms in chickens, depending on virus dosage and age of infected chicken.

Water starvation in poults. Research at Beltsville has shown that extremely high mortality in young turkey poults may result from rapidly drinking large amounts of water following water deprivation periods. Mortalities of 60 to 100% were obtained when two-week-old poults were deprived of water for approximately 48 hours. Attempts to return water-starved poults gradually to full water were unsuccessful. These results indicate that early mortality in poults may be due, in some cases, to water starvation instead of pathogens and emphasize the need for supplying drinking water to young poults at all times.

Drug-resistance of coccidia. Coccidiosis is the most important intestinal parasitic disease of poultry and is a serious disease in livestock. It has been found that the species of coccidia that cause cecal coccidiosis in chickens gradually acquired resistance to every drug tested. It was also found that in building resistance to one drug, the coccidia may also increase its resistance to another. This indicates that outbreaks of coccidiosis in flocks already receiving a drug will not be controlled by use of another drug. Development of such cross-resistance, however, doesn't necessarily work both ways. For example, coccidia exposed to one drug A, gain resistance to it and to an untried drug B, but if the coccidia are exposed initially to drug B, and develop resistance to it, they may remain susceptible to drug A until exposed to it long enough to build resistance. This work emphasizes the need for a continual search for new drugs for the control of coccidiosis.

Influence of environment on disease. Research at Wisconsin has been carried out to determine how the environment which poultry are subjected to may influence their susceptibility to disease. This work has led to the discovery that levels of ammonia in air commonly found in poorly ventilated poultry housing renders birds more susceptible to infection with respiratory disease viruses than birds not exposed to this contaminated air. This work may explain why poultry disease problems such as airsacculitis are much more prevalent under conditions of poor management.

Ventilation of livestock buildings. Research in cooperation with State Experiment Stations has obtained much needed basic data on the heat and moisture given off by cattle, hogs, and poultry, and on the influence of building environment on production and feed consumption. The heat and moisture dissipation data are considered basic design data for ventilation systems of poultry, dairy, and swine buildings. They appear in design handbooks including the 1964 Guide and Data Book of the American Society of Heating, Refrigeration, Ventilating and Air Conditioning Engineers, and are used by makers of ventilating equipment, prefabricated buildings and package buildings as well as by specialists advising farmers on their own construction. Building improvements resulting from the above research have contributed to the substantial rise in efficiency of livestock production that has occurred during the past decade.

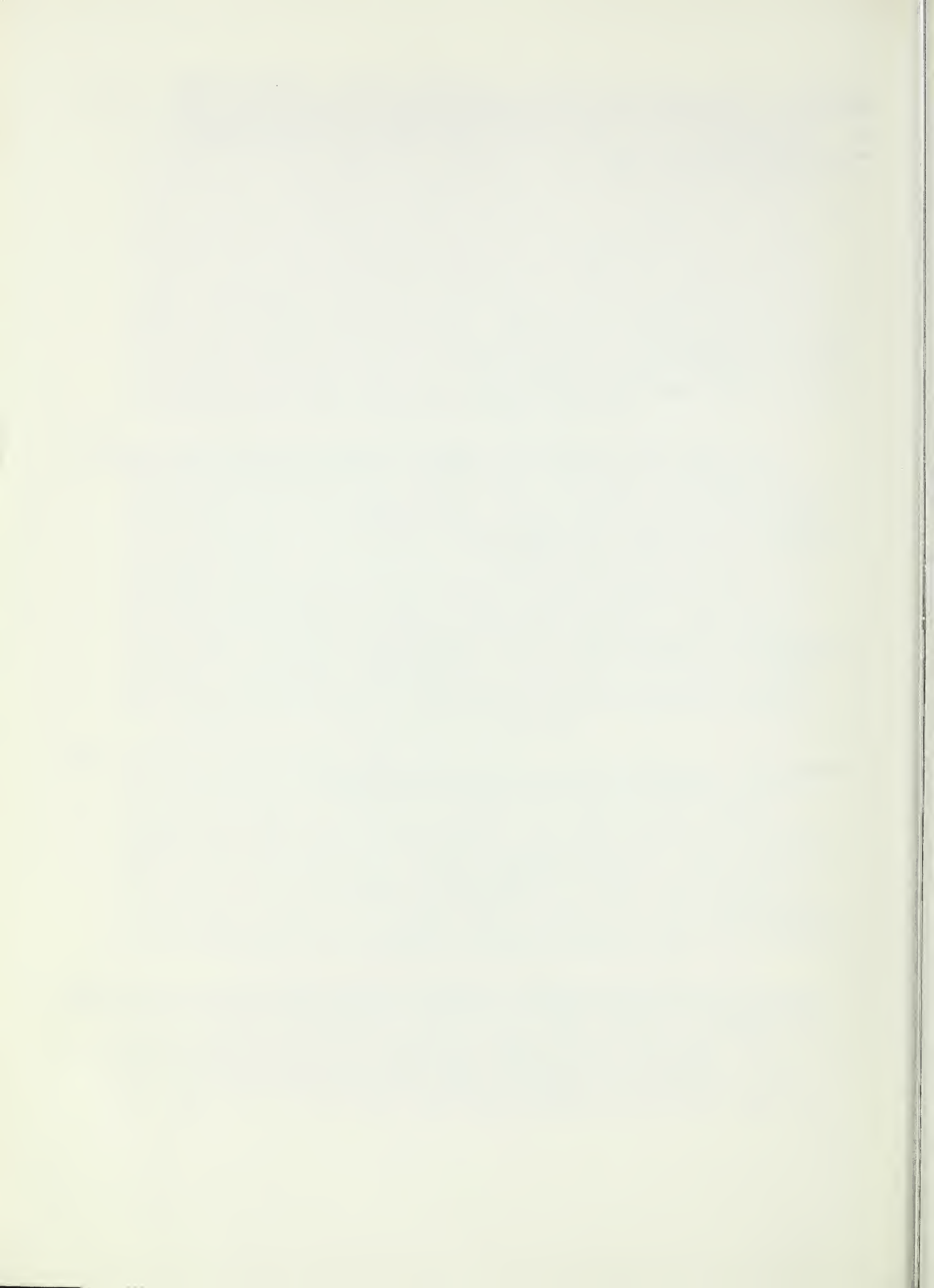
Process developed for destroying Salmonella in liquid egg white. A process has been developed by Department scientists for stabilizing liquid egg white so that it can withstand pasteurization temperatures of 140-143° for 3-1/2 minutes--a condition necessary for destruction of Salmonella. Previously, adequate pasteurization was not possible because the necessary high temperatures cause coagulation of the egg white. The new process entails treatment of egg white with trace amounts of an edible aluminum salt to a concentration of 30 parts per million. Such stabilized pasteurized egg white yields angel cakes of volume and texture equivalent to those prepared with fresh eggs. Successful commercial runs with the new process have been made in two egg processing plants. Nearly 200 million pounds of egg white with a market value of about \$25 million are produced annually in the U.S. The development of this pasteurization treatment is an important step toward the elimination of Salmonellosis food poisoning outbreaks that can be attributed to egg products.

Radiation preservation of poultry meat. Department scientists have defined processing factors that minimize deleterious changes in radiation-sterilized poultry meat. The research, supported by funds transferred from the Quartermaster Research and Engineering Command of the Department of Defense, demonstrated the importance of heating poultry to about 180° F. prior to irradiation. In addition to preventing enzymatic reactions resulting in off-flavor, this heat treatment eliminates an unattractive red discoloration which otherwise develops during storage of irradiated poultry. Additional improvement in flavor was accomplished by irradiation at subfreezing rather than ordinary temperatures. As a result of these and other findings, the QMC has concluded that radiation-sterilized poultry meat is a suitable product for feeding troops in locations where refrigeration is not available or economical.

New facilities for handling meat and poultry in New York City. As a result of studies by the Transportation and Facilities Research Division, ARS, an additional \$40 million complex of facilities is being planned for handling meat and poultry, for which the New York City Board of Estimate has allotted \$6.1 million for site acquisition and design. The facilities are being planned adjacent to the new \$36 million fruit and vegetable facility that is under construction at Hunts Point and will replace the 14th Street and Brook Avenue Markets. Total annual saving in handling fruits, vegetables, meat, and poultry in new facilities is estimated to be almost \$25 million.

Experimental in-line egg cleaner minimizes spoilage hazards and breakage. An experimental in-line egg cleaner, developed under a research contract with the University of California, has recently been field-tested successfully in a commercial plant by ARS personnel. The tests in a commercial plant indicate that cleaning effectiveness is more than 70 percent better than commercial cleaners now in use while the

breakage rate and spoilage hazard is greatly reduced. Based on the assumption that approximately 20 percent of the 5 billion dozen eggs sold annually off U. S. farms undergo some type of cleaning treatment, industry-wide use of the new cleaner could result in millions of dollars saved annually.



I. FARM RESEARCH

POULTRY - BREEDING

Animal Husbandry Research Division, ARS

Problem. Poultry breeders have made tremendous progress in recent years through the application of new genetic principles revealed by basic research. Improvement in many of the economic traits has attenuated and new methods of releasing useful genetic variation are needed. Information is needed as to the relative rates of progress which will result from various breeding systems for improving such economic traits as egg and meat production. Furthermore, information is needed as to whether different breeding systems are required or are more efficient at different stages of the breeding program. In order to design the most efficient breeding systems, knowledge of the heritabilities, genetic correlations between traits and the effects of genotype-environment interactions are required. The physiological basis for the action of certain genes is unknown and information regarding these processes would lead to better control of heredity for optimum performance. The economics of production should be improved through knowledge gained on the genetic aspects of feed utilization and selection under various stress conditions.

USDA AND COOPERATIVE PROGRAM

This is a continuing long-term program involving basic and applied studies of the inheritance of egg production and broiler characteristics. Scientists with majors in genetics or biochemistry and minors in physiology or statistics are involved. Much of the research is conducted within the framework of four regional projects. In addition to major contributions to the establishment and maintenance of central facilities, the USDA also provides coordinating personnel located at Athens, Georgia; Lafayette, Indiana; and Beltsville, Maryland. The close working relationship between the USDA and State experiment stations in the four regional projects provides for integrated research on a large scale without duplication of effort. Research at Beltsville, Maryland, involves the selection of lines under stress of nutritional deficiency and for differences in feed utilization efficiency, including a study of genetic, biochemical and physiological differences between these lines. Selection for response in egg production to "18-hour" days is conducted in cooperation with AERD. Research in the North Central region is on egg production traits and is done at the Regional Poultry Breeding Laboratory, Lafayette, Indiana, and at 12 cooperating State experiment stations. In the Southern region the emphasis is divided between egg and broiler traits and the work is conducted at the Southern Regional Poultry Genetics Laboratory, Athens, Georgia, and at 14 cooperating State experiment stations. The work in the Northeastern region involves the improvement of chickens through genetic and physiological studies and is conducted under cooperative projects at 11 cooperating State experiment stations. Cooperative work on turkeys is carried on with six Western States.

A Research and Marketing Act contract with Purdue University Agricultural Experiment Station for the study of diallel crosses of four light and four heavy type inbred lines was concluded. The study extended over a period of three years and involved a statistical analysis of data obtained from inbred lines of poultry and their combination in crosses from the North Central Regional Poultry Breeding Project.

The Animal and Poultry Breeding Department, Ministry of Agriculture, Dekki, Giza, Egyptian Region, U.A.R., is conducting a study to improve and evaluate the Fayoumi and Dandarawi fowl. This P.L. 480 project was initiated in 1963 for a duration of five years. Other P.L. 480 projects initiated during the year include a pilot project designed to study the influence of environmental stress with *Tribolium*, to be conducted by the Instituto Nacional de Investigaciones Agronomicas, Madrid, Spain, and a project to evaluate the bacteriological problems in artificial insemination of hens to be conducted by the Hebrew University, Faculty of Agriculture, Rehovot, Israel. The latter two projects are scheduled for a duration of five and two years respectively.

A total of 7.3 professional Federal man-years is devoted to this program annually. Of this number 3.6 man-years are devoted to genetics and inter-relations of performance traits, 3.1 to selection and systems of breeding and 0.6 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

Research is concerned with estimating genetic parameters, gene-environmental interactions and genetic and phenotypic correlations among morphological and physiological traits of economic significance in chickens and turkeys. These estimates are used for evaluation of breeding systems.

Much of the breeding research is conducted within the framework of four Regional poultry breeding projects, NC-47, S-57, NE-51 and W-78, in co-operation with the USDA. Work on NC-47 is designed to determine rates of progress obtainable in populations of chickens under various systems of breeding and to estimate genetic changes produced by these systems. S-57 is concerned with establishing the magnitude and importance of genotype-environment interactions in meat and egg production stocks. The pleiotropic effects of known genes in relation to reproductive performance of chickens is studied in NE-51. W-78, a turkey breeding project, is concerned with efforts to improve hatchability of turkey eggs at high altitudes through selection for hatchability at different altitudes.

Considerable effort is being devoted to studies of genetic traits that are associated with reproductive fitness, fertility and hatchability particularly in turkeys. Many genes in poultry have multiple effects and the relationship of plumage color genes, blood group genes and other easily

identified marker genes to growth rate, viability, egg production and other economic traits is being investigated. Studies are being made to identify genetically-controlled physiological functions for possible use as selection criteria to improve growth and reproductive efficiency; to determine the ability of chickens to make genetic adaptations to atypical light-dark cycles and to determine if selection for growth rate is more effective using deficient rations as compared with adequate rations.

The total State scientific effort devoted to poultry breeding research is 50.6 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Genetics and Interrelations of Performance Traits.

1. Genetic aspects of nutritional deficiencies. Selection for fast and slow growth on both a methionine deficient diet and on a normal diet for two generations indicates that lines differing in growth rate can be developed under each environment. Progress has been made from selection in divergent directions under each environment when compared with the randombred control population. Chicks reared on the deficient diet were restricted in growth at 3-weeks of age to approximately one-half that of chicks on the normal diet. When fed a normal diet from 3- through 8-weeks of age the chicks did not compensate for the reduced growth rate observed during the treatment period. (AH el-48)

2. Biochemical basis for genetic differences in growth rate. Lines of chickens established by selection for differential growth performance on adequate and methionine deficient diets are being used to investigate the nutritional basis of the genetically-induced growth rate alteration. Although the fast growing lines are much heavier than the slow growing lines at 3 weeks of age, the methionine and protein requirements of all 4 lines are similar. Using a conventional growth assay, the optimal dietary concentrations were found to be .39% and 18.4% for methionine and protein, respectively. Additional evidence that no differences in nutrient requirements existed among the lines was provided by studies involving the quantitation of feed intake. Both fast growing lines were more efficient in the conversion of feed than were the slow growing lines. However, when birds of the fast growing lines were fed the same quantity of feed as that consumed by the slow growing lines, no difference in growth was noted. The data indicates that selection for growth rate on a methionine deficient diet is accomplished through genetic differences in appetite and not through changes in requirements. (AH el-52)

3. Selection for egg production under sub-circadian periodicities. Data obtained from the second generation of White Leghorns selected for egg production under 18-hour "short days" differed only slightly from that obtained from the initial generation. Percent hen-day egg production was

1.3% greater than the previous year and 3% above the randombred controls. The percent hen-day production of the 24-hour control population was 1.8 and 2.3% superior to the previous generation and the randombred controls, respectively. Differences noted in the second generation between the 18-hour and control population were small for all traits except 8-week body weight and age at first egg. Birds in the 18-hour population were 40 grams heavier at 8 weeks and matured 11 days later than those in the 24-hour control population. Two light treatments (constant and step down) were superimposed during the growing period on the 18-hour population during the first two generations. Differences were small and not significant for percent hen-day production, 20-week body weight and 320-day body weight. Eight-week body weights were higher for the step down group, while birds reared on the constant photoperiod matured 7 days earlier. A study utilizing the Coturnix quail under the same two environments was initiated during the year to determine if these birds respond to selection programs in a manner similar to chickens. The data available after the completion of the first generation, from the two 144 bird populations, serves only as an indication of the comparative performance of birds maintained under the 18-hour cycle as opposed to the 24-hour controls, since no genetic selection has been introduced up to this point. The hen-day egg production over the 90-day laying period was 70 and 67% for the control and 18-hour population, respectively. This difference was not significant. (AH el-47)

4. Genotype-environment interactions. Genotype-environment interaction studies with both egg and meat production stocks were conducted as a part of the Southern Regional Poultry Breeding Project. Data analyzed to date indicate that the performance of both meat and egg production stocks is influenced by interactions of genotype and the environment. Further analysis of the data is being completed to assess the magnitude and importance of the interactions relative to the total observed variation. (AH el-44)

5. Genetic variation in chemical or physiological traits. Selection for resistance and susceptibility to gonadotropic hormone inhibition in lines developed from the Regional Cornell Control was continued at the Southern Regional Poultry Genetics Laboratory. Data from the first selected generation were analyzed to estimate the heritabilities and the genetic and environmental relationships of inhibition and production traits. Results indicate that the heritability of days of inhibition estimated from the sire component of variance was approximately 0.30. The corresponding value for pretreatment egg production was 0.21. Genetic correlation estimates between the two traits were approximately -.49, indicating that indirect selection for resistance to inhibition would not be as efficient in selecting for egg production as selection for the trait directly. (AH el-50)

Following six generations of selection at the Maryland Station for high and low serum cholesterol at six weeks of age the cholesterol level of the high line was approximately thirty percent greater than that of the low line. The heritability of serum cholesterol level was calculated to be .25 in a

randombred population. The high cholesterol line had lower juvenile weight and lower blood pressure, but higher egg albumen quality, than did the low line. There were no consistent, statistically significant, differences between the lines with respect to productive traits or in yolk cholesterol. Liver synthesis of cholesterol was equivalent in the two lines but fecal excretion of the metabolite was greater in the low line. (AH el-45)

Positive and negative selection at the New Jersey Station for blood pressure in two lines of chickens produced a difference of 56 between males of the two lines and 44 between the females after five generations of selection. Levels of blood pressure in the two lines was not associated with mortality among the males but mortality was greater in hypotensive than in hypertensive females. A similar situation was found with respect to the abilities of the birds of the selected lines to withstand exercise stress. In general, the biological efficiency of the birds was better in the high pressure line than in the low pressure population. The heritability of systolic blood pressure was estimated at approximately .27. (AH el-45)

6. Genetic variation in economic traits. At the Georgia Station comparisons were made between two populations of randombred controls differing in genetic base. Results from the second generation of divergent selection for 8-week body weight in each population indicate that the total response between the high and low lines of the narrow base population was 93.1% of the progress made in the broad base population. Similar results were observed in generation one. (AH el-44)

In a test at the New Hampshire Station of recurrent versus closed flock selection for improving broiler qualities there was no evidence that a cross-line progeny test had any advantage over a pure line progeny test for improving 8-week body weight within a line. Cross-bred progeny of 4th generation males from the two selected lines mated to randombred females were heavier from the recurrent line than from the closed line but not significantly so. (AH el-45)

In selection experiments at the Oregon Station with Broad Breasted Bronze turkeys, first generation low libido line males were less responsive in individual and group comparisons of aggressive mating behavior than the high libido and random lines males. During the first four weeks of egg production the receptivity of high libido line females was higher than for females of the other two lines. In twenty 30-minute observation periods during the four weeks, high libido line females completed an average of 3.5 matings per hen as compared to 1.8 and 1.9 for the low libido and random lines females. The fertility following natural matings with random line males averaged 59.5, 39.6 and 44.7% for the high libido, low libido, and random lines. With later matings by artificial insemination within lines the eggs averaged 93.3% fertile, with no differences between lines. Body weight and conformation measurements indicated there were no differences between lines due to selection for libido. (AH el-46)

7. Genetics of shell pigmentation in Japanese quail. An autosomal recessive mutation which, in the homozygote, results in almost complete lack of egg shell color has arisen in the colony of Japanese quail (*Coturnix coturnix japonica*) maintained by Poultry Physiology Investigations. All F₁ female progeny of white-egg hens mated to randombred normal males lay normally pigmented eggs. Matings of F₁ males and females produce normally pigmented and white-egg females in a 3:1 ratio. Backcross matings of F₂ white-egg females to F₁ males produce normally pigmented and white-egg female progeny in a 1:1 ratio. Males homozygous for the white-egg trait have been identified by test matings of male backcross progeny with white-egg females. A purebred white-egg breeding colony, producing 50-75 progeny weekly, has been established. The usefulness of the Japanese quail as an experimental animal has been broadened by the establishment of the white egg strain. Pedigree markings on the white shell can be read with far less chance of error, and accurate staging by candling can now be accomplished for quail embryos as easily as for chick embryos. Considerable interest in obtaining the white egg strain has been expressed by a number of geneticists, physiologists, and virologists throughout the United States. (AH e3-22)

B. Selection and Systems of Breeding.

1. Evaluation of genetic changes produced by various breeding systems. The evaluation of breeding systems and selection methods was continued at five stations and at the central facilities of the North Central Regional Poultry Breeding Laboratory. Three randombred control populations are maintained and made available to cooperators as foundation stock and controls. Eggs from these control stocks are shipped to many locations throughout the country upon request. Selection at each station is for one trait, hen-day percent egg production to about 300 days of age. Other traits are recorded but are not the basis for selection. Selection pressure is maintained at approximately 25% for both male and female progeny.

At the Indiana Station, closed flock index selection and reciprocal recurrent selection in Cornell White Leghorns and Purdue Pool populations were continued and crosses of the index and reciprocal recurrent selection populations were tested. Two generations of selection have been tested. Results of these tests indicate little or no gains in hen-day percent egg production from selection based on individual, sire and dam family means (index) or reciprocal recurrent selection.

At the Kansas Station, a study of index and reciprocal recurrent selection was continued on foundation stocks of Cornell White Leghorns and Rhode Island Red Regional Controls, and their crosses. After three generations of selection, all index selected populations were above the controls when comparisons were made based on deviations of selected populations from controls. Reciprocal recurrent selected populations showed no increase

over the controls. Unselected traits generally showed little change from the controls.

The Missouri Station has completed four generations of selection for percent egg production using both recurrent selection to inbred testers and index selection. The females used in both of these breeding methods and also the males in the index selection were from the randombred control populations. The inbred tester lines used as the male parent in the three recurrent selection populations consisted of a White Leghorn, a White Plymouth Rock and a Rhode Island Red line. A White Leghorn flock selected on the basis of an overall performance index is designated as the intra-flock population.

The intraflock population and crosses had essentially the same hen-day percent egg production from first egg to 64 weeks of age, as the selected randombreds. The above populations exhibited slightly better production than the randombred controls. After four generations, two of the recurrent selection lines show no indications of an increase in production, while in one recurrent selection line production is somewhat higher.

Twelve inbred lines, of Regional Cornell Control stock, were continued at the South Dakota Station through 1963. Six of these lines (three selected and three random) have provided sires for pullets which are currently being tested at three substation locations. At a fourth location, one of the selected inbred lines provided sires for one of the five pens of test pullets. The performance of pullets from selected and from random inbred sires were compared with each other and with other mating types in the 1962-63 tests. At one location the pullets from selected inbred sires performed better than did the purebreds and those from random inbred sires. Age at maturity, mortality, egg size, and adult body weight were similar for the three groups. Broodiness and maturity varied somewhat at the different stations.

Samples of 45 populations with three replications of each were compared, under standard environmental conditions, at the North Central Regional Poultry Breeding Laboratory. Sixteen economic traits were measured on 3,700 birds. The Regional Cornell Randombreds, on which 17 selection systems are based, remained relatively stable in all traits at the completion of six generations of random mating. Ten of the systems were superior to the base population in hen-day percent egg production; however, none of these differences reached statistical significance at the 5% level. The sire family selection system with half and full-sib matings was significantly lower than the controls as expected. The sire family selection system with restricted inbreeding and the dam family selection system were not different from the controls. The individual selection system was below the controls, but the difference was not statistically significant. In general, index selection using individual, sire and dam family records ranked high when compared to the other methods of selection; however, most of the effects of selection occurred in the first generation. For the

first time, the selected populations generally ranked below the controls in egg weight. This may be due to a reversal in the downward trend in egg weight that had previously been shown by the control populations. (AH el-43)

An analysis of diallel crosses of four light and four heavy type inbred lines was conducted by the Purdue Station under a Research and Marketing Act contract. The results indicate that cross x year interactions were not important in the inbred lines, but contributed significant effects in the single and double crosses. The presence of a cross x year interaction reduced the efficiency of predicting single and multiple cross performance. In general, the variation due to general combining ability effects among the crosses was larger and more important than reciprocal or specific combining ability effects. The prediction of single cross and top cross performance from knowledge of the inbred lines was low when multiple R^2 values were used, whereas parent-offspring regressions suggest that adequate predictions are feasible. (Research and Marketing Act Contract No. 12-14-100-5763 (44)).

2. Randombred control populations. Five randombred control populations are maintained for use as genetic and environmental controls and as a gene pool for use in initiating new research. Three stocks are maintained at Lafayette, Indiana, primarily for egg production research and two at Athens, Georgia, for meat production research. Hatching eggs from these populations are supplied to research workers at experiment stations, to random sample tests and to commercial poultry breeders. (AH el-43, AH el-44)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Genetics and Interrelations of Performance Traits

- Becker, W. A. 1963. Pre-incubation storage of turkey eggs and its effect on body weight. Poultry Sci. 42:1356-1359. (AH el-46)
- Becker, W. A. and Bogyo, T. P. 1963. Heritability of developmental time and viability of turkey embryos in three environments. Proc. XI Int. Cong. Genetics, Vol. 1:153-154. (AH el-46)
- Bogyo, T. P. and Becker, W. A., 1963. Exact confidence intervals for genetic heritability estimated from parental half-sib correlations. Biometrics 19:494-496. (AH el-46)
- Cook, R. E., Hess, C. W., and Dembnicki, E. F. 1963. Importance of genotype-environment interactions in egg production stocks. Poultry Sci. 42:1261-1262. (AH el-44)
- Friars, G. W., Bohren, B. B. and McKean, H. E. 1963. Time trends in estimates of genetic parameters in a population of chickens subjected to multiple objective selection. Poultry Sci. 4:1773-1784. (AH el-43)
- Gutierrez, J. H. 1963. Genetic and physiological responses of the domestic fowl to progesterone treatment. M.S. Thesis, University of Georgia. (AH el-44)

- Harris, P. C. and Wilcox, F. H. 1963. Studies on egg yolk cholesterol.
1. Genetic variation and some phenotypic correlations in a random bred population. Poultry Sci. 42:178-182. (AH el-45)
- Harris, P. C. and Wilcox, F. H. 1963. Studies on egg yolk cholesterol.
2. Influence of season. Poultry Sci. 42:182-185. (AH el-45)
- Harris, P. C. and Wilcox, F. H. 1963. Studies on egg yolk cholesterol.
3. Effect of dietary cholesterol. Poultry Sci. 42:186-189. (AH el-45)
- King, S. C., VanVleck, L. D. and Doolittle, D. P. 1963. Genetic stability of the Cornell randombred population of White Leghorns. Genetic Res. 4:60-74. (AH el-45)
- Marks, H. L. and Lucas, L. M. 1963. Time of oviposition under "short days." Poultry Sci. 42:1466-1468. Abstract. (AH el-47)
- Poole, H. K. 1964. Egg shell pigmentation in Japanese quail. Genetic control of the white egg trait. Journal of Heredity, Vol. 55:136-138. (AH e3-22)
- Saadeh, H. K. 1963. Non-additive genetic variation, maternal effect and genotype x environment interaction effect as estimated from the body weight of chickens. Unpublished M.S. thesis. University of New Hampshire Library, Durham, New Hampshire. (AH el-45)
- Van Vleck, L. D., King, S. C. and Doolittle, D. P. 1963. Sources of variation in the Cornell Controls at two locations. Poultry Sci. 42:1114-1125. (AH el-43)
- Weinland, B. T., Carson, J. R., and King, S. C. 1964. Stability of gene frequencies in a randombred control population of chickens. Poultry Sci. 43:985-990.
- Wilcox, F. H., Chermis, F. L., Jr., VanVleck, L. D., Harvey, W. R. and Shaffner, C. S. 1963. Estimates of genetic parameters of serum cholesterol level. Poultry Sci. 42:37-42. (AH el-45)
- Wilcox, F. H., Shaffner, C. S. 1963. Performance of lines selected for high and low cholesterol, Poultry Sci. 42:1033-1035. (AH el-45)

Selection and Systems of Breeding

- Abplanalp, H., Ogasawara, F. X., Asmundson, V.S. 1963. Influence of selection for body weight at different ages on growth of turkeys. British Poultry Science 4:71-82. (AH el-46)
- Morgan, W. and Kohlmeyer, W. 1963. Inbreeding with and without selection. Genetics 48:902. (AH el-43).
- Ogasawara, F. X., Abplanalp, H. and Asmundson, V. S. 1963. Effect of selecting turkeys for increased body weight on reproduction in turkey hens. Poultry Sci. 42:838-842. (AH el-46)

POULTRY - PHYSIOLOGY
Animal Husbandry Research Division, ARS

Problem. Continuing basic research in avian physiology is essential to establish fundamental concepts and to increase the knowledge upon which ultimately must depend the solution of such problems as fertility, hatchability, growth and egg production. Basic physiological knowledge is necessary also for implementation of the subject matter of other disciplines. In reproductive physiology, for example, the dominant role of the central nervous system is now generally recognized, but much intensive research will be required before we can expect any "useful" knowledge of mechanisms by which the varying actions of external and internal factors are integrated and directed to initiate, maintain or modify reproductive functions. Many aspects of environmental physiology, of responses to stress, and of growth and development likewise depend upon basic research. On the more immediately practical side, increased knowledge of poultry housing, related equipment and other management factors is necessary to provide optimal ranges of operational efficiency.

USDA AND COOPERATIVE PROGRAM

This is a continuing program, mainly on basic aspects of the physiology of avian reproduction, but including also applied studies pertaining to environmental physiology and management. In addition to physiologists, the work draws upon geneticists and animal husbandmen. Research is in progress at Beltsville, Maryland, and Glendale, Arizona, the work at Glendale contributing to regional project W-50. Cooperation currently is maintained with members of the Farm Electrification Branch, AERD; Bellevue Hospital, New York City; the National Institutes of Health; and Pennsylvania State University. Federal research in this area calls for 7.4 professional man-years, distributed to subareas as follows: Physiology of reproduction, 4.4; environmental physiology, 2.1; physiology of growth and development, 0.5; and program leadership, 0.4.

PROGRAM OF STATE EXPERIMENT STATIONS

Research is concerned with the basic aspects of the physiology of growth, reproduction, fertility and hatchability in chickens and turkeys as well as applied aspects pertaining to environmental physiology and management. Fundamental research is in progress to elucidate the role of the nervous system and the endocrine system in controlling ovulation and oviposition in poultry. Investigations are being conducted on the physiology of sperm in order to develop techniques for storage and to dilute semen without reducing fertilizing capacity for use in artificial insemination.

The states of the Western region and the USDA are cooperating through Regional project W-50 to study the effect of different combinations of light, temperature, altitude and other environmental factors on

reproductive performance of the chicken. Some stations are investigating the effect of light and temperature on thyroid gland function, the influence of wave length of light upon growth and production, the effect of amount of light per day or light intensity during the growing period on subsequent reproductive performance in the female.

The field of poultry behavior is being investigated and includes such activities as imprinting, cannibalistic behavior, sexual behavior and social organization of the flock.

Consideration is given to the influence of male hormones on embryonic development of the bursa of Fabricius as it affects antibody production of chickens in the immediate and future generations.

Investigations into embryonic mortality and hatchability in relation to growth and development are underway. These studies are concerned with the effects of embryonic irradiation exposure upon subsequent life span, the establishment of optimum level of gases in the incubator atmosphere for normal embryonic development and investigation of mechanisms by which the embryo utilizes the components of the egg.

Research is underway on the circulatory system and involves physiological studies on the heart and factors concerned with blood pressure regulation.

The total State scientific effort devoted to poultry physiology research is 46.6 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Physiology of Reproduction.

1. Neuroendocrinology. Work was continued with the chicken on the effects of mechanical stimulation of the brain on oviposition. Any of several brain manipulations causes premature lay of the terminal egg of a recurrent 2-egg sequence to occur within a specific 2-hour period on the morning of normal lay. This almost certainly means that the time of premature lay is governed by diurnal periodicity in some undetermined physiological state of the hen. Preliminary efforts were made to establish some of the factors involved in the phenomenon of diurnally restricted premature lay.

Current fragmentary knowledge of the control of oviposition indicates that lay results from the abrupt release of neurohypophyseal hormone(s). An initial experiment was therefore undertaken to establish whether premature lay resulted from an earlier release of hormones from the posterior pituitary. Following the development of a routine useful technique for neurohypophysectomy, the effects of removal of the posterior pituitary on normal and induced oviposition of the terminal egg were compared. Surprisingly, elimination of the posterior lobe did not delay or prevent

normal lay of the terminal egg (C_t) of the cycle, nor of C_t lay induced by brain stimulation. The results suggest two mutually exclusive conclusions: (1) Neurohypophyseal hormones play no essential role in oviposition in chickens or (2) the posterior pituitary serves only as a non-essential storage depot for the effective hormone and all of the basic machinery for the production and release of this hormone is present in the components of the hypothalamo-hypophyseal system proximal to the posterior lobe. The first premise would be extremely difficult to test by available experimental methods. The second possibly, however, can now be tested by classic physiological techniques applied to the neurohypophysectomized hen.

Penultimate ovipositions of the hen's sequence are generally thought to be subject to controls which are lacking or inoperative during lay of the terminal egg. To test the possible influence of these factors on premature lay, a comparison was made of the effects of brain stimulation on the first and last ovipositions of the 2-egg sequence. Basic differences were noted in the type of mechanical stimulation required to induce lay, in the times of effective stimulation, and in the hours of premature lay. Premature C_t lay could be induced by the insertion of electrodes into the cerebral cortex, median thalamus or preoptic brain, but C_1 lay (first egg of the cycle) could be forced only by insertion of preoptic electrodes. As tested by placement of electrodes in the preoptic brain, stimulation during the period of 9 P.M. to midnight on the night before lay proved most effective for premature C_1 lay, while stimulation during the hours of 6-9 A.M. on the morning of expected lay was most effective for C_t lay. Nearly all premature C_1 ovipositions occurred between 6 and 8 A.M., while premature C_t lay, as in earlier experiments, was restricted to the hours of 10 A.M. to noon. The physiological basis for the observed difference in response between the first and last eggs of the sequence remains to be elucidated. Evidence from other sources indicates that factors associated with release of ovulating hormone from the pituitary and/or ovulation are involved.

It has been proposed recently that the processes of urine excretion and egg shell formation compete for calcium and that this is the basic cause of reduced shell thickness in the summer months. According to the theory, increased water consumption in hot weather leads to increased urine output, thereby reducing the amount of calcium available for shell formation. The theory was tested in neurohypophysectomized chickens held under stable conditions of temperature and humidity. Neurohypophysectomy causes severe polydipsia and polyuria, but does not permanently interfere with egg production. In this experiment shell thickness in eggs laid by neurohypophysectomized hens did not differ significantly from shell thickness of eggs laid by the same hen before lobectomy even though water consumption and urine excretion were increased by 2 to 5 times the preoperative levels. The results offer strong evidence that some temperature related factor other than urinary excretion of calcium is responsible for reduced shell thickness. (AH e3-21)

The hen's coupled ovulation cycle includes a single day of lapse between ovulations or sequences of consecutive daily ovulations. The uncoupled cycle follows a lapse of two or more days. In coupled cycles, the release of gonadotrophic hormone (GTH) for ovulation of the terminal follicle (C_n) of one cycle appears also to evoke maturation of the first or C_1 follicle of the succeeding cycle. As the number of members (n) in coupled cycles increases from 1 to about 7, the release of GTH for maturation of the C_1 follicle occurs at progressively later hours. On theoretical grounds, it seemed improbable that the time of GTH release for maturation of the C_1 follicle of uncoupled cycles could be subject to factors believed to account for the progressively later times of release appearing in coupled cycles. Examination of the conditions under which the uncoupled GTH release takes place led to the surmise that release might occur during a diurnally recurrent "critical period" falling at about the hour of the C_1 maturation release in lengthy coupled cycles. If this were so, it seemed likely that first ovipositions of uncoupled cycles of low n would occur somewhat later in uncoupled than in coupled cycles, and that the difference would decrease as n increased. Times of ovipositions were therefore compared in coupled and uncoupled cycles of 1, 2 and 3 members. In a selected group of White Leghorn hens, first ovipositions were found to occur 46 minutes later in uncoupled 1 member cycles, 19 minutes later in 2 member cycles and 4 minutes later in 3 member cycles. These differences, and notably the order of differences, are in accord with the view that the neural component of the GTH release mechanism exhibits, at the uncoupled C_1 maturation release, a 24-hour periodicity or "critical period" which appears in a constant relationship with some phase of photoperiod. It is suggested that the varied patterns of timing seen in GTH release cycles are imposed upon this fundamental 24-hour periodicity by feedback hormones of ovarian origin.

2. Parthenogenetic reproduction. Parthenogenetic development in chicken and turkey eggs is characterized by its slowness in getting underway once the eggs are placed within the incubator. The underlying cause for this delay is believed to represent the time required for highly disorganized cells to regroup so as to form a normal blastoderm. To get more precise information on the degree of variability existing among parthenogenetically developing eggs, the following tests were conducted.

Newly laid, unfertilized turkey eggs were incubated at 99.3° F. and 100 eggs were removed and broken after having been incubated 24, 48, 72, 96, 120 and 144 hours, respectively. Each blastoderm was examined macroscopically and data collected on the growth (diameter) attained by each developing disc.

Much variation was found among eggs of each age group in the numbers and sizes of developing blastoderms. Only one of the 100 eggs examined at 24 hours of incubation had a developing blastoderm. The marked change occurred between 24 and 48 hours of incubation, during which time 35 eggs showed evidence of parthenogenetic development. There were no significant changes in the number of developing blastoderms during subsequent periods of

incubation. Twenty-seven, 44, 41, and 27 developing blastoderms were encountered in eggs at 72, 96, 120, and 144 hours of incubation, respectively.

Variability in sizes of developing blastoderms within groups became more pronounced as incubation progressed. For example, 21 developing blastoderms at 96 hours and 11 at 120 hours of incubation had attained diameters equivalent to 12 to 24 hours normal development. However, developing blastoderms of other eggs within these same age groups were far more advanced, having attained stages of growth at which membranes covered the entire surface of the yolk. Several of these more advanced blastoderms had given rise to parthenogenetic embryos about the size of 2 or 3 day normal embryos.

A cooperative study with Dr. Frank Raucher, NIH, was initiated to determine (1) if dissociated parthenogenetic cells of turkey eggs could be induced to develop on the chorio-allantoic membranes of chicken and turkey eggs and (2) if these embryonic cells still retain the ability of giving rise to embryos. The possibility of parthenogenetic embryos arising from individual cells was suggested by studies of twinning, in which a high incidence of multiple embryos were encountered in unfertilized turkey eggs.

Turkey embryonic cells of parthenogenetic origin, on being dissociated with trypsin were placed on the chorio-allantoic membranes of 10 day chicken and turkey embryos and injected into the body cavities of 4 day old turkey poults. No embryoid-like structures were found following the inoculation of eggs or poults. (AH e3-19)

3. Homograft reactions. Last year's report presented evidence that histocompatibility antigens can be present in skin grafts from male turkey parthenogens which are not present in their progeny by unrelated females, and it was concluded that these parthenogens were heterozygous at segregating genetic loci assumed to control expression of histocompatibility antigens. However, the possibility remained that a "maternal effect" could have been responsible for the incompatibility since the parthenogenetic sires and their progeny had different dams. Subsequently, two parthenogens were mated, each to his own dam, and produced seven and four backcross progeny, respectively. A wattle skin graft was transplanted from each sire to each of his own progeny. Four of seven and two of four backcross progeny rejected their respective sires' skin. Grafts to the remaining progeny have survived permanently. These results are additional strong evidence of heterozygosity at histocompatibility loci in turkey parthenogens and appear to exclude the possibility that "maternal effects" were the cause of the graft rejections reported previously. (AH e3-20)

4. Estrogen-modified sex in turkeys. Three hundred micrograms per egg of estradiol benzoate in corn oil were injected into Beltsville Small White turkey eggs on the second day of incubation. Controls included uninjected and corn oil injected eggs. Hatched poults were autopsied at hatching 4, 6,

16, and 35 weeks of age. No morphological abnormalities were observed in the control groups at any age. Embryonic and posthatching mortality was high in the estradiol injected group. Effect on genetic females was generally one of hyperfeminization, specifically hypertrophy of Mullerian ducts. Intersexes were noted at all ages. The combined number of intersexes and normal males approximated the number of females and led to the assumption that the intersexes were genetic males. They exhibited a range of modifications from small, cystic testes or complete gonadal suppression (agonadal birds) to formation of an ovotestis. Mullerian ducts were present in varying degrees of development in some but not all intersexes. The observations indicated initial severe feminization of genetic males followed by either gonad suppression or regression toward genetic sex type (ovotestis formation). There was no evidence of complete or permanent sex reversal with this estradiol benzoate treatment.

B. Environmental Physiology.

1. Water starvation in poults. In June 1963, heavy losses were sustained in young Beltsville Small White poults after they had drunk water avidly following failure to find their drinking water during an estimated period of about 48 hours. No pathology was found in the dead birds. In a series of experiments following this loss, it was shown that, after water deprivation periods of 48 to 52 hours, the rapid ingestion of water was followed promptly by mortalities of 60 to 100% in poults 11 or 18 days of age. Following a deprivation period of 24 hours, mortality in 18-day old poults was 40%, but poults 11 days of age were unaffected as were poults 3-1/2 to 11-1/2 weeks old subjected to waterless periods of 24 to 52 hours. Attempts to return water-starved poults gradually to full water consumption were unsuccessful. These findings suggest a possible explanation for some of the mortality that occurs frequently in young poults in the absence of any gross pathology. They also emphasize the need for care in making changes in the method of supplying drinking water to young poults to insure continued consumption without long periods of deprivation. (AH e3-18)

2. Controlled photoperiods in chickens. Experiments have continued at Glendale, Arizona, on the effects of controlled light of various patterns during the growing period on subsequent egg production of October-hatched White Leghorns. Regardless of the light patterns and regimens used during the growing period, age at sexual maturity was delayed and egg size remained greater with a decrease from natural light. With an increase over natural light, sexual maturity was hastened and egg size remained smaller. Using a step-down light pattern during the growing period and a step-up pattern during the laying period resulted in delayed sexual maturity and larger egg size, but effects on total egg size were contradictory. (AH e3-16)

3. Comparison of caged and floor-housed pullets. At Glendale, Arizona, individually caged pullets laid slightly fewer eggs and experienced slightly greater mortality than did pullets maintained on litter floors. Pullets housed 5 in 24 by 18 inch cages laid fewer eggs than did birds housed

individually in 8 or 10 by 18 inch cages. Mortality, due mainly to vent picking, averaged 34 percent for group caged pullets against 8 percent for individually caged birds. Cutting back both mandibles or including a tranquilizer in the diet did not appreciably lower mortality in the pullets housed 5 to a cage. In comparing population density on litter floors, birds maintained one per 3 square feet produced a few more eggs with lesser mortality than did those maintained on 1-1/2 square feet each. Pullets on slat floors laid substantially fewer eggs and consumed more feed than did birds on litter. Both eggs and feed were lost through the slat floors. (AH e3-17)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Physiology of Reproduction

- Fraps, R. M. 1964. Follicular maturation and diurnal periodicity in the hen's ovulation cycle. Proc. Endocrine Soc., 46th Meeting, p. 87. (Abs.)
- Opel, H. 1964. Premature oviposition following operative interference with the brain of the hen. Endocrinology, 74, 193-200. (AH e3-21)
- Poole, H. K. 1963. Some effects of estradiol benzoate on development of the reproductive system of the turkey. British Poul. Sci. 4, 279-284. (AH e3-20)
- Poole, H. K. 1963. Observations on the chromosomes of turkey-chicken hybrids. J. Heredity, 54, 101-102. (AH e3-20)

Environmental Physiology

- Lowe, R. W. and Heywang, B. W. 1964. Effect of various light treatments during the growing period on egg production of October-hatched White Leghorn pullets. Poultry Sci. 43, 11-15. (AH e3-16)
- Marsden, S. J. and Lucas, L. M. 1964. Effect of short-day or low-intensity light treatments on reproduction of fall-hatched turkeys in two environments. Poultry Sci. 43, 434-441. (AH e3-18)

POULTRY - NUTRITION
Animal Husbandry Research Division, ARS

Problem. The goal of nutrition research is to amass information so that poultry diets may be formulated and fed to produce the best quality product at the least possible cost. The problem logically divides into two areas: (1) furnishing the nutritive requirements of poultry, and (2) the feedstuffs that supply these requirements. A refined methodology is needed to estimate more accurately the energy (carbohydrates and fats), protein (amino acids), vitamin and mineral requirements of poultry of various ages, strains and levels of production. Even more urgently needed is information on the relationships that exist between these nutrients, if the formulation of optimum nutritive balance in diets is to be attained. Additional information is required on the effect of feed additives (antibiotics, arsenicals, hormones, enzymes, antioxidants, tranquilizers) on nutritive requirements, and on the utilization of protein and energy. Somewhere in the maze of requirements, interrelationships and interactions, it must be determined which portion of the diet is for intestinal microorganisms and which is for the host. Also, the vast field of interrelationships between disease and nutrition remains to be explored. In the feedstuffs area, how much of a particular nutrient that is present should be known, but of more importance is how much is available to the bird. Thus, information on digestibility, absorption, chelation and interactions is necessary. In addition, the complete composition of a feedstuff must be known. At the present, the proximate analysis is the only information available about major dietary constituents; consequently, the nutritionist does not know exactly what is being fed when a diet is formulated. There may be present growth promotant and/or inhibitors of which he is not aware.

USDA AND COOPERATIVE PROGRAM

This is a continuing program conducted by nutritionists on basic and applied research on the nutritive requirements and digestion and metabolism of poultry and the nutritive value of feedstuffs. The work is in progress at Beltsville, Maryland, and at the Southwest Poultry Experiment Station, Glendale, Arizona. Some phases of work at Glendale are carried on in cooperation with the Departments of Biochemistry and Poultry Science of the University of Arizona at Tucson. Studies concerned with the influence of different factors on the metabolism of vitamin A in chickens are in progress at the Hebrew University, Israel. Its duration is for three years, 1962-1965, and involves PL 480 funds. (A10-AH-7)

A project for the evaluation of the protein quality and energy values of feedstuffs available in India is in progress at Punjab Agricultural University, Ludhiana, India. Its duration is for five years, 1964-1969, and involves PL 480 funds. (A7-AH-21)

The Federal effort devoted to research in the poultry nutrition area totals 6.7 professional man years. Of this number 2.0 are devoted to digestion and metabolism, 1.6 to nutritive value of feeds, 1.7 to protein and energy requirements, 1.0 to other nutritive requirements, and 0.4 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

Research is being conducted on the chicken and turkey requirements for specific amino acids, minerals, vitamins, energy and unidentified growth factors for maintenance, growth, egg production and health.

The mineral studies are concerned with trace mineral requirements and the interrelationship of calcium, phosphorus and vitamin D associated with bone calcification and egg shell quality. The detrimental effect of high levels of dietary calcium on hatchability of turkey eggs is being studied. The influence of specific polyunsaturated fatty acids on egg size and cholesterol metabolism as well as protein and energy requirements and interrelationships are being investigated. In addition to the establishment of requirements, vitamin research is concerned with the role of vitamin E in encephalomalacia and vitamin K in blood clotting mechanisms.

The effect of alfalfa, anticoagulants and vitamin K on the incidence of blood spots in eggs is being studied. Only a limited amount of research is being conducted on unidentified growth factors. Sources of xanthophyll pigments are being evaluated as economical sources of broiler pigments. Studies are being conducted on the nutritive value of locally grown feed-stuffs and methods of increasing the availability of starch and protein by chemical, physical and enzymatic treatment. New antibiotics are being evaluated for growth promoting ability, but only limited research is being conducted on the mode of action of antibiotics on growth promotion.

Management. Intensified methods of production are resulting in changes in management practices. Broiler studies are concerned with the effect of heat stress and floor space on growth rate and feed efficiency. The reproductive performance of chickens as affected by light or feed restriction during the growing period, housing density in floor pens or cages and temperature stress are being investigated. Studies on floor, feeder and water space needs and litter and housing practices for efficient turkey production are being conducted.

The total State scientific effort devoted to poultry nutrition and management is 55.4 man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Nutritive Requirements.

1. Vitamin requirements. Studies at Glendale, in cooperation with the

University of Arizona, in which White Leghorn pullets were fed stabilized vitamin A at levels ranging from 250 to 3,000 USP units per pound of diet showed that 1,250 USP units were adequate for maximum production, feed conversion, fertility and hatchability. Adequate vitamin A intake during growth has considerable influence on subsequent performance since pullets raised on minimum levels did not produce as well as those raised on adequate levels. The lowest incidence of blood spots in eggs was found at the highest level of vitamin A intake.

Experiments were conducted at Glendale and the University of Arizona to determine the vitamin A requirements of chickens grown in a subtropical, semiarid climate. The results indicate that when vitamin A was increased from 500 to 3,000 USP units per pound of diet there was no significant improvement in growth rate and feed conversion to 10 or 20 weeks of age. In all of the tests there was an increase in vitamin A stored in the liver as the level of the vitamin in the diet was increased. Chickens reared during the cooler periods stored more vitamin A in the liver than those reared during hot weather. Studies at Glendale in cooperation with the University of Arizona showed that sodium ascorbate fed to White Leghorn layers at levels of 10, 20 and 454 mgs. per pound of feed, had no appreciable effect on egg weight, shell thickness, or ratio of dried shell weight to whole egg weight. (AH e2-15)

2. Mineral requirements. In continuation of the study of calcium and phosphorus requirements of broiler chickens, a fourth test was conducted in an effort to refine the requirement values and, in particular, to determine with more accuracy the upper level of the range of calcium adequacy. The trial was with mixed sexes, and involved calcium levels of 0.75, 0.80, 0.85, 0.90 and 0.95%, and total phosphorus levels of 0.6, 0.65, 0.70 and 0.75%. The results indicate no significant difference in growth response to calcium levels between 0.8 and 0.95% on all phosphorus levels. It was clearly indicated that phosphorus levels of 0.70% and above resulted in depression in growth rate and decrease in efficiency of feed utilization. The results of the four studies, involving over 25,000 chickens, show that under practical conditions, the optimum dietary levels of these minerals for maximum performance are 0.80-0.85% calcium and 0.60-0.65% total phosphorus. This phase of investigation of mineral requirements is completed. (AH e2-18)

3. Fat requirements. In continuation of studies concerned with the effect of linoleic acid deficiency on reproductive performance, Leghorn females were reared on a linoleic acid-free diet. At maturity, the pullets were distributed into six groups and groups one through six were maintained on the deficient diet, plus 0, 10, 20, 40, 80, or 250 mg. linoleic acid/day/hen, respectively. Egg production, egg size, and hatchability paralleled the increase in dietary linoleic acid with the exception of zero hatchability for groups 1 and 2. Egg production, egg size, and hatchability in group 6 did not equal that of hens on a practical diet, indicating that 250 mg. linoleic acid/day/hen was insufficient for optimum

performance. There is an indication that the severe depletion resulted in irreparable damage to the hens, or that the diet was lacking in some nutrient other than linoleic acid. When the depleted hens received linoleic acid equal to that in the practical diet, egg production, egg size and hatchability increased, but after a two-month period were not equal to that of the practical diet. No linoleic acid could be detected in the yolk fat of eggs from hens maintained on linoleic acid-free diet. It was present only in trace quantities in the yolk fat of eggs from hens receiving 40 mg. or less of the acid/hen/day after 20 weeks. As the linoleic acid content of the yolk fat increased there was a concurrent decrease of the C-20 triene.

In experiments to determine the fat and fatty acid requirement of Coturnix quail, it was found that the growth rate of quail on a purified isolated soy protein diet was improved by the addition of corn or safflower oil; however, linoleic acid failed to increase growth rate equivalent to that of the intact oils. The purified diet supplemented with oil did not support growth comparable to that obtained with practical diets. The linoleic acid content of the tissues of quail on diets deficient in this fatty acid were reduced, but not to the same extent as comparable tissues of all chicks deficient in linoleic acid. (AH e2-13)

B. Digestion and Metabolism.

1. Metabolism of fats. Studies to determine the effect of fat depletion of laying hens on the growth rate and fatty acid composition of their progeny were conducted. Depletion of the hens for 16 weeks did not affect the growth rate of the progeny, but after the hens had been depleted of essential fatty acids for 32 weeks the growth rate of their progeny was significantly lower than the progeny of hens fed fat in their diets. Essential fatty acid depletion of these hens resulted in a reduction of the level of polyunsaturated fatty acids and an increase in monounsaturated fatty acids in the plasma and heart fat of the progeny. During the depletion period, the linoleic acid levels decreased more rapidly than the arachidonic acid level in the plasma and heart fat of the progeny. The chicks from hens on the fat-free diet were late in hatching, and had a lower level of linoleic and arachidonic acid and a higher level of C-20 triene than chicks from hens receiving dietary fat.

Experiments concerned with increasing the hatchability of eggs from linoleic acid deficient hens by injection of linoleic acid or methyl linoleate into the yolk resulted in no improvement in hatchability. This indicates that the developing embryo is unable to utilize free fatty acids.

Studies to determine the effect of dietary linoleic acid deficiency on the reproductive performance of male chickens showed that the lack of this fatty acid resulted in decrease in semen production and in fertility. Fatty acid analysis of the semen showed a low linoleic acid content compared to other tissue for both linoleic acid deficient and normal males. The semen

of normal males contained about ten times as much linoleic acid as that from the deficient males. Two unidentified fatty acids were observed, one of which was much higher in the deficient males, and the other higher in the normal males. (AH e2-13)

2. Utilization and function of vitamin A. Studies on the function and utilization of vitamin A at the Hebrew University, Rehovoth, Israel, indicated that feeding acidulated soybean soapstock increased the liver storage of vitamin A in chicks. This was not due to improved utilization of performed vitamin A, but to the presence of a carotenoid having pro-vitamin A properties. The pigment was characterized by a single absorption maximum of 460 m μ and has a biological activity of approximately 0.4 I.U. per microgram. It has been shown that lutein is the major precursor of the pigment which is formed by treating lutein, or lutein containing materials with strong acids. The pigment has been tentatively identified as 3-hydroxy -3, 4-dehydro-beta-carotene, which is known to be formed from lutein by the loss of one mole of water.

Investigation of the influence of thiouracil and thyroxine on carotene and vitamin A utilization indicates that thiouracil does not hinder carotene conversion and that thyroxine definitely enhances the utilization of dietary vitamin A. (A10-AH-7)

C. Nutritive Value of Feeds.

1. Effect of feeding cottonseed meal. In continuation of cottonseed meal studies at Glendale, in cooperation with the University of Arizona, seven samples (representing glanded and glandless meals prepared by different processes) were tested for their effect on coloration of stored eggs. There were some discolored yolk in some of the eggs stored 15 days when several of the five glanded meals were fed, and after six months' storage, practically all the yolks of eggs produced from feeding these five meals were discolored. One of the glandless meals caused discolored yolks after six months' storage; whereas, the other glandless meal had no effect on the yolks. Since pink whites were present in eggs resulting from feeding four of the five hexane extracted meals, it is apparent that hexane does not remove all components that cause pink whites. (AH e2-17)

2. Nutritive value of grains. Tests to determine the feeding value of corn, wheat, oats, and barley were conducted with laying hens, using protein levels of 10, 12.5, and 15 percent. The diets were isocaloric for each protein level, and only one grain was used as a carbohydrate source per protein level. The results indicate that all of the grains supported egg production at approximately the same level on the 12.5 and 15 percent protein diets. On the 10 percent protein diet, oats gave 60 percent production, as compared with about 44 percent with the other three grains. (AH e2-14)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Nutritive Requirements

- Lillie, R. J., Twining, P. F., and Denton, C. A. 1964. Calcium and phosphorus requirements of broilers as influenced by energy, sex and strain. Poultry Sci. (In press) (AH e2-18)
- Twining, P. F., Lillie, R. J., Robel, E. J., and Denton, C. A. 1964. Calcium and phosphorus requirements of broiler chickens. Poultry Sci. (In press) (AH e2-18)

Digestion and Metabolism

- Menge, H., Miller, E. C., and Denton, C. A. 1964. Effect of a fat-free maternal diet on the fatty acid composition of the progeny. Poultry Sci. 43: 164-168. (AH e2-13)
- Menge, H., Calvert, C. C., and Denton, C. A. 1964. Further studies on the effect of a low-fat diet on reproduction in the hen. Poultry Sci. (Abstract). (AH e2-13)

Nutritive Value of Feeds

- Heywang, B. W., Kemmerer, A. R., and Lowe, R. W. 1963. Discoloration in eggs when cottonseed meal feeding was stopped before the pullets laid. Poultry Sci. 42: 995-997. (AH e2-17)
- Kemmerer, A. R., Heywang, B. W., Vavich, M. G., and Phelps, R. A. 1963. Further studies of the effect of cottonseed oil on discoloration of cold storage eggs. Poultry Sci. 42: 893-895. (AH e2-17)
- Heywang, B. W., Reid, B. L., and Kemmerer, A. R. 1964. Effect of sodium ascorbate on egg shell thickness during hot weather. Poultry Sci. 43: 625-629. (AH e2-15)

POULTRY - IMPROVEMENT OF VIABILITY
Animal Husbandry Research Division, ARS

Problem. Leukosis continues to head the list of diseases that cause a high mortality among chickens. It is prevalent in both young and mature stocks, and on most, if not all, farms where chickens are reared. It is also responsible for high rates of condemnation at packing plants and poor performance with respect to growth and egg production.

The yearly financial losses to the poultry industry of the United States from leukosis mortality alone are estimated to be in excess of \$65,000,000. At this time when the margin between the cost of production and the price received for poultry products has been reduced to a very low figure, it is more urgent than heretofore that additional emphasis be placed on research directed towards the development of effective control measures for the leukosis complex.

USDA AND COOPERATIVE PROGRAM

This is a continuing program conducted by virologists, pathologists, and a geneticist on basic and applied studies of the neoplasms of the avian leukosis complex. Most of the studies are conducted at the Regional Poultry Research Laboratory, East Lansing, Michigan. Work is also done in cooperation with more than 15 other Federal, State, or commercial agencies located in several sections of the United States.

The primary objective of these studies is to develop a practical program for the prevention of losses due to the diseases of the avian leukosis complex. Most of the effort has been and currently is directed towards two approaches. The first is to develop a program to increase resistance of the chickens to avian leukosis. It has been found that the level of viral stimulated antibodies and certain yet undefined physiologic mechanisms are genetically controlled and have very important influences on resistance. For significant progress in this approach, it has become evident that basic studies must continue to be conducted on (1) mode of inheritance, (2) mechanism of gene expression, (3) interrelationships of resistance to different tumor viruses, avenues of exposure, and other genetically controlled traits, (4) dynamic interrelationship between infection, antibody, and neoplasms, (5) ultrastructure, biochemistry, and the dynamic molecular biology of the causative virus and infected cells, and (6) the mechanism of influence of the bursa of Fabricius on neoplasia.

The second approach is directed towards the prevention of infection and/or elimination of the disease. Avian leukosis is a contagious disease. The infectious virus is transmitted not only by direct contact with infected chickens and with infected environment, but also via the infected embryonating egg. The most important prerequisite for progress in the development of eradication measures is a simple but adequate method of detecting

current or past infection. Only recently has notable progress been made on this aspect and further simplification of procedures can be expected. Such fundamental epizootiological information as the extent and prevalence of infection, the modes of spread of infection, the importance of various vectors and reservoirs, the importance of various environmental factors and other concomitant diseases, and the influence of passive as well as active immunity must be obtained before a rational program of eradication can be developed.

Recent developments have demonstrated that there are at least two different families of viruses--not one, as formerly thought--that cause similar types of leukosis. This emphasizes the magnitude of the problem that still faces us.

A cooperative project entitled "Studies on the epizootiology of avian lymphomatosis and related neoplasms" calls for the active cooperation of (1) National Cancer Institute, NIH; (2) the Animal Disease Eradication Division, ARS; and (3) the American Poultry and Hatchery Federation. Also cooperating in this project are poultry industry representatives located in Michigan and Indiana. Furthermore, cooperative projects are in effect with the Alabama and New Jersey Experiment Stations; the Pennsylvania Department of Agriculture; and the School of Medicine, University of Minnesota. The Southern and North Central Poultry Breeding Projects and poultry breeding farms in California and Connecticut are also cooperating in research with the Regional Poultry Research Laboratory at East Lansing. A research contract at the Wisconsin Agricultural Experiment Station has been completed and the final report will be available for the next report.

The Federal scientific effort devoted to the research in this area totaled 11.9 professional man-years. Of this number, 3.0 are devoted to studies of causative agent, 2.0 to epidemiology, 1.3 to improvement through genetics, 1.5 to immunology and vaccination, 2.0 to virus propagation and tissue culture, 0.7 to management practices and 1.4 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

The genetic mechanisms of resistance and susceptibility to disease are being investigated. This research is concerned with the relationship of blood group antigens to inheritance of disease resistance and the mode of gene action controlling resistance to viruses of the avian leukosis complex. Research is being continued on selective breeding practices as a means of controlling poultry diseases.

Studies are being made on changes in physical characteristics of chicken blood that may be related to susceptibility to respiratory disease viruses. The extent to which hormones of the various endocrine glands affect resistance to disease is being investigated. Consideration is also given to the relationship of nutrition to disease resistance. Metabolic studies are being conducted to determine those nutrients that affect resistance

of chicks to viral infections.

The total State scientific effort devoted to Improvement of Viability in poultry is 1.1 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Studies of the Causative Agent.

Studies of viral strains causing primarily neurolymphomatosis have continued. Transmission with the JM strain can easily be effected in line 7 chickens by inoculation with cell suspensions and direct contact with infected birds, but disease induction with cell-free filtrates has been disappointing. Other sources of virus such as the B14 strain of Houghton, England, the Hiawassee field isolate, and the line 7 RPL isolate have given similar results. Problems which must be resolved before critical results can be obtained with this type of virus include (1) the development of a flock of line 7 chickens free of this type of virus infection, (2) provide the type of high security facilities capable of containing the more contagious neural disease virus, and (3) develop better standards for quantitating inoculation response since gross or even microscopic examination does not identify all diseased birds.

Studies on the oncogenic potential of tumor viruses revealed that a standard preparation of Rous sarcoma virus at high dilution causes visceral lymphomatosis and erythroblastosis. These neoplasms have now been found to be caused by a leukosis virus associated with Rous virus which other workers have shown to act as a helper virus in the production of infectious Rous virus particles.

In two tests, mouse leukemia viruses were inoculated into susceptible line 151 embryos and day-old chicks. In the first test, 19 of 177 chickens developed one or more leukotic neoplasms, but in a more extensive second test where 4 different strains of virus were used, only 2 of 493 chickens showed leukosis lesions. Potent avian leukosis virus (strain RPL 12) also failed to induce disease in leukemia susceptible mice. These experiments provide no evidence that the leukosis viruses are capable of crossing the avian-murine interspecies barrier.

Ablation of the bursa of Fabricius has been found to have a profound effect in reducing the incidence of visceral lymphomatosis in chickens inoculated with strain RPL 12. In contrast, the removal of all thymus tissue had no detectable effect. The removal of either organ had no effect on the occurrence of erythroblastosis, osteopetrosis, or other neoplasms. The marked reduction in occurrence of visceral lymphomatosis in bursectomized birds was obtained even though time of inoculation varied from 1 to 28 days at bursectomy from 1 to 56 days of age. (AH e6-10)

Since the RIF test for the detection of leukosis virus is dependent on the transformation of chick embryo cell cultures by the Rous sarcoma virus, it was important to determine the cause of resistance to transformation found in embryos of some of the isolated line 15I population. Several attempts to detect or isolate a resistance inducing virus have failed. In addition, 121 hens of 2 generations of this population did not have antibody to a leukosis virus.

In the testing of embryos from individual sire-dam matings by both the tissue culture and chorio-allantoic membrane methods, it was determined that the lack of sensitivity in line 15I embryos was genetic and probably due to the single recessive gene, rs. Other studies showed that this type of resistance is present in other stocks of chickens and should be taken into consideration when conducting RIF tests directly on embryo cell cultures. (AH e6-3 and AH e6-24)

In collaborative studies with Drs. Ziegel and Rauscher of the National Cancer Institute, it was found that the virus which occurs naturally in the pancreas of many embryos and young chicks is in fact a visceral lymphomatosis virus. Pancreatic extracts compare well with strain RPL 12 with respect to induction of leukosis and causing a high rate of infection of embryos and chicks resulting in virus multiplication in the acinar pancreatic tissue.

Studies in cooperation with Dr. Levine of American Cyanamid Co. provide strong evidence that the resistance inducing virus from their flock of chickens is in fact leukosis virus. Virus isolated from embryos of two different sources was propagated through 11 and 12 serial tissue culture passages. The last passage supernatant was inoculated into line 15I chickens and a high incidence of neoplasms of the avian leukosis complex (ALC) resulted. (AII e6-24)

B. Immunology and Vaccination.

Six additional experiments on attempts to develop an effective killed vaccine have been completed. It was found that 0.4% formalin for 5 days or 0.5% betaprone for 2 days inactivated all the virus in a 10% tumor extract stored at 2°C and maintained at a pH of 7.0-7.4. With storage for 17 days, 0.2% formalin was sufficient to result in complete inactivation. Treatment with ethyl ether and tween caused complete inactivation, but no immune response was obtained even when Freund's complete adjuvant was used.

Using preparations of strains RPL 12 or RPL 29 virus treated as above, it has been found that good neutralizing antibody levels were obtained only when sufficient live virus was present in the vaccine to cause deaths from leukosis. When live virus was absent, the vaccinated chickens did not develop antibody during an eight-month period even though good adjuvants were given with the vaccine. This failure may be due to an insufficient amount of inactivated viral antigen injected even though the infectivity

titer of the source material was quite high.

Results on the study of the antigenic character of ALC viruses indicate that many of them can be used as a "helper" virus with defective Rous sarcoma virus (RSV) in producing fully infective viruses having the antigenic character of the "helper" virus. Seven specific helper - RSV strains have been produced. These include three "laboratory" strains, RPL 12, RAV-Rubin, and strain A, and four recently isolated field strains. These new RSV strains produce typical focal areas of altered cells in cultures of chick embryo fibroblasts. Potency determinations have been made and antigenic character studies are now in progress. Preliminary results indicate that antigenic analyses of field isolates and/or serum analyses from field outbreaks can be made and relationships determined between viruses causing these outbreaks and the standard stock strains.

Studies on the complement fixation test for avian leukosis (COFAL) viruses indicate that it is comparable in sensitivity and specificity to the RIF test. Of 187 comparable tests conducted, an agreement of 98% has been obtained. Also, there is an indication that the COFAL test may be adapted to detect tumor viruses which do not show RIF activity.

The COFAL test is shorter, simpler, and less expensive than the RIF test. Sufficient high titer complement fixing hamster anti-Schmidt-Ruppin RSV serum has been produced to do approximately 50,000 COFAL tests.

Studies on the relationship of virus dose, age of host, route of inoculation and neoplastic response to the immune response are in progress. The results demonstrate important interrelationships. Doses which induce a neoplastic response also induce an immune response, and conversely in dose lots which do not produce neoplasms, the incidence of the immune response is very low or absent. (AH e6-17)

C. Epidemiology.

In the 1952-53 Pennsylvania Random Sample Test, mortality due to avian leukosis complex (ALC) varied among entries from 0 to 21%. This is an indication of variation in susceptibility and/or variation in congenital and acquired infection. Even though there was considerable mortality before and after the birds were 5 months of age, there was no correlation among the entries between early and late ALC mortality. No correlation was found between the presence of maternal antibody, nor acquired antibody measured at 338 days, and the incidence of neoplasia. By 338 days in the 1962-63 test and 150 days in the 1963-64 test, all entries tested were almost 100% Rous sarcoma virus antibody (RSVA) positive. The rate of mortality in the 1963-64 test has been very low.

A cooperative study with the New Jersey Agricultural Experiment Station has demonstrated the importance of contact transmission and rearing on recently used litter on the incidence of ALC. Isolated 151 chickens on clean litter

had a lower mortality than those in contact with commercial chickens or those reared on dirty litter. The commercial chickens reared on contaminated litter had the highest ALC mortality.

In a cooperative study with the National Institutes of Health and Klager Hatcheries, over 1500 blood samples from chickens of 13 flocks in Southern Michigan were tested. The percent with maternal RSVA varied from 30 to 93%. There was a great variation within the same flock on different samplings taken at monthly intervals. March had the lowest mean incidence of RSVA positives of 56.3%, and February the highest of 78.7%. There is an overall repeatability of 80 to 92% (depending on criteria used) with the RSVA test and a positive correlation of 0.8 between the RIFA and RSVA tests. Thus the simpler and more rapid RSVA tests is sufficiently accurate for epizootiologic studies. Ninety-nine mammalian sera have been tested for RSVA. Five human sera have shown low anti-RSV activity and 2 have shown high activity. Both the latter came from patients with acute juvenile stem cell leukemia. None of 30 bovine and canine sera showed activity. (AH e6-27)

D. Improvement through Genetics.

Through the use of artificial insemination and individual cages, the number of individual male matings for each inbred line has been increased. The second generation of full-sib matings under this system has resulted in information on reproductive performance indicating that selection among full-sib mated families may result in fairly reproductive inbred lines. The progress of inbreeding is being followed with continued skin grafting and blood typing work in cooperation with the Alabama Experiment Station. (AH e6-2, AH e6-28)

The existence of a single autosomal dominant gene (Rs) which influences in vitro as well as in vivo susceptibility to Rous sarcoma virus (Bryan strain) has been fully confirmed. Preliminary studies suggest that the homozygous resistant (rs rs) animals or cells do not support virus growth as well as susceptible (Rs rs; Rs RS) ones. (AH e6-24, AH e6-29)

Efforts to evaluate the usefulness of inoculation with tumor virus preparation as a method of selection for resistance to lymphomatosis indicate that at least some virus isolates rank a series of matings of different inbred lines differently than others. This and other evidence suggests that resistance to avian tumor viruses is specific to the particular virus isolate used, rather than general resistance to all the viruses of the avian leukosis complex. These studies have been extended to include four field locations where the same series of matings are exposed naturally in all locations and inoculated at the Regional Poultry Research Laboratory. (AH e6-29)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Studies of the Causative Agent

- Burmester, B. R. and Fredrickson, T. N. 1964. Transmission of virus from field cases of avian lymphomatosis. I. Isolation of virus in line 151 chickens. J. Nat. Cancer Inst. 32: 37-63 (AH e6-10).
- Dmochowski, L., Grey, C. E., Padgett, F., Langford, P. L., and Burmester, B. R. 1964. Submicroscopic morphology of avian neoplasms. VI. Comparative studies on Rous sarcoma, visceral lymphomatosis, erythroblastosis, myeloblastosis, and nephroblastoma. Texas Reports on Bio. Med. 22: 20-60 (AH e6-24)
- Peterson, R. D. A., Burmester, B. R., Fredrickson, T. N., Good, R. A. 1963. Prevention of lymphatic leukemia in the chicken by the surgical removal of the bursa of Fabricius. J. Lab. Clin. Med. 62: 1000 (AH e6-10)
- Peterson, R. D. A., Burmester, B. R., Fredrickson, T. N., Purchase, H. G., and Good, R. A. 1964. Effect of bursectomy and thymectomy on the development of visceral lymphomatosis in the chicken. J. Nat. Cancer Inst. 32: 1343-1354 (AH e6-10)
- Piraino, F., Okazaki, W., Burmester, B. R., and Fredrickson, T. N. 1963. Bioassay of fowl leukosis virus in chickens by the inoculation of 11-day-old embryos. Virology 21: 396-401 (AH e6-3, AH e6-24)
- Zeigel, R. B., Burmester, B. R., and Rauscher, F. J. 1963. An electron microscopic study of the chick embryo pancreas following natural and artificial transmission of avian tumor viruses. J. Cell. Biol. 19: 76A (AH e6-24)

Immunology and Vaccination

- Fredrickson, T. N. and Burmester, B. R. 1964. Responses of different stocks of chickens to inoculation as embryos and as chicks with strain RPL 12 and field isolates of leukosis virus. Avian Diseases 8: 123-134 (AH e6-24)
- Purchase, H. G., and Okazaki, W. 1964. Morphology of foci produced by standard preparations of Rous sarcoma virus. J. Nat. Cancer Inst. 32: 579-589 (AH e6-24)

Improvement Through Genetics

- Crittenden, L. B. 1963. A simple method of skin grafting in chickens. Poultry Sci. 42: 1398-1399 (AH e6-28)
- Crittenden, L. B., Johnson, L. W., and Okazaki, W. 1964. Histocompatibility and erythrocyte antigen variability within highly inbred lines of White Leghorns. Transplantation 2: 362-374 (AH e6-28)
- Crittenden, L. B., Okazaki, W., and Reamer, R. 1963. Genetic resistance to Rous sarcoma virus in embryo cell cultures and embryos. Virology 20: 541-544 (AH e6-24, AH e6-29)

POULTRY - BROILER LOSSES
Animal Husbandry Research Division, ARS

Problem. Effective control of Air Sac Disease to reduce the continuing losses from condemnations is a major problem of the broiler industry. Since the presence or absence of pathogenic strains of the pleuropneumonia-like organism, *Mycoplasma gallisepticum*, largely determines whether chickens will develop Air Sac Disease in the presence of secondary invaders, such as *E. coli*, Newcastle disease or infectious bronchitis, the value of effective methods for control is evident. Additional basic information is needed concerning the behavior of the organisms associated with this disease complex and the host response to them. The great range in host response indicates that genetic variation, nutrition, environment and management play a part in the severity of the response in individual flocks of chickens.

USDA AND COOPERATIVE PROGRAM

A basic and applied program of research directed toward the reduction of losses from broiler condemnations is to be conducted jointly by specialists in agricultural engineering, animal diseases and poultry management, genetics, nutrition, and physiology. Two locations are involved in this work, the Southeast Poultry Research Laboratory, Athens, Georgia, and the South Central Poultry Research Laboratory, State College, Mississippi.

The Animal Husbandry Research Division's work at Athens emphasizes genetics and physiology in relation to the Air Sac Disease complex and the work at State College will emphasize environment and management in relation to condemnation losses.

This research program is cooperative with the Animal Disease and Parasite and Agricultural Engineering Research Divisions, ARS. Local cooperation of State experiment stations and the broiler industry in the southeast and south central regions is an important part of the program, particularly with respect to field trials.

The Federal effort devoted to research in this area totals 3.2 professional man-years. Of this number 1.0 is devoted to management practices, 0.5 to equipment and facilities, 1.0 to genetic control, 0.5 to environmental physiology, and 0.2 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

The States are engaged in basic and applied research investigations on the effects of adverse environmental factors on broiler losses. Genetic and physiological aspects of environmental stresses are being investigated. Studies are being made on the effect of such adverse conditions as; sudden chilling, prolonged chilling and overheating prior to or during the

brooding period on rate of mortality, rate of growth and extent of condemnation losses. Consideration is being given to other environmental factors as housing, density, type of housing, air movement, air contamination, litter management and sanitation.

The total State scientific effort devoted to Environment as related to broiler losses is 4.7 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Genetics in Relation to Airsacculitis.

In an effort to control egg transmission of Mycoplasma gallisepticum, hatching eggs from a randombred population were immersed in Tylosin tartrate for 30 minutes at the Southeast Poultry Research Laboratory. The chicks hatched from these eggs were reared in isolation and in several small lots. No reactors to the serum plate test were found up to 13 weeks of age, when the birds were transferred from batteries to floor pens. These birds will be used as a base population in an attempt to produce lines resistant and susceptible to the organisms responsible for airsacculitis.

B. Management in Relation to Condemnations.

Comparisons were made in field trials of zinc baciferm and aureomycin prestarters. There were 138,602 chickens started in three series of trials on seven different farms. The differences between the two prestarters varied with farms. The growth and feed conversion were improved enough by either prestarter to increase the margin over feed cost over that of the untreated controls.

On-the-farm studies of avian nephrosis (Gumboro Disease) in broilers have been conducted. None of the various preventive methods and treatments studied have been consistently effective for avian nephrosis. Treatments included sulfonamides, antibiotics, vitamins and molasses. House treatments included litter removal, new litter, disinfectants, and lye. When used with security management, lye and a synthetic phenolic disinfectant showed some promise. Mortality studies involved 2,060,528 chicks started in 209 houses on 28 infected farms. Field trials included 851,482 chickens started on the 28 farms. (AH e7-1)

At the Southeast Poultry Research Laboratory blood samples were collected from broiler field trials totaling 143,350 birds. These birds consisted of both PPLO-free and non-PPLO-free stock. Limited data indicate that when chicks from PPLO-free and positive parentage were reared together, the condemnation rate was significantly higher among the PPLO-free lots than when this stock was reared separately. Also, it appears that environmental stress during the summer is not sufficient to greatly increase the condemnation rate of PPLO-positive stock.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Management in Relation to Condemnations

- Parkhurst, R. T. 1964. Pattern of mortality in avian nephrosis. Poultry Sci. 43: 788-790. (AH e7-1)
- Parkhurst, R. T. 1964. Avian nephrosis (Gumboro Disease) in U.S.A. broilers: Treatment trials. World's Poultry Sci. Jour. 20:3 208-211. (AH e7-1)
- Parkhurst, R. T. 1964. On-the-farm studies of Gumboro Disease. (In press) (AH e7-1)

POULTRY PERFORMANCE AND MANAGEMENT RECORDS
Animal Husbandry Research Division, ARS

Problem. Livestock and poultry improvement cannot be accomplished effectively without adequate records of performance and management. Furthermore, records which have widespread utility must be produced through carefully coordinated programs in order that uniformity may be obtained in measurements and analytical procedures. Continual revision of record procurement and evaluation techniques in accordance with current research findings requires integration of program operations and research. Only in this way can there be a continual chain of discovery, application, and field testing.

USDA AND COOPERATIVE PROGRAM

This is a continuing long-term program of performance testing dairy cattle and poultry, including the evaluation of the genetic merit of dairy cows, sires and herds, chickens for egg or meat production, and turkeys. Also included in the program is the control of hatchery disseminated poultry diseases. The work on dairy cattle performance testing is cooperative with 50 States and Puerto Rico and the Records and Breeding Committees of the American Dairy Science Association. Cooperation is also carried out with the National Association of Artificial Breeders and the various dairy cattle breed registry organizations. The poultry work is cooperative with Official State Agencies in 47 States and with the supervisors of 23 random sample tests in the United States and Canada.

The Federal scientific effort devoted to the programs in this area totals 6.5 professional man-years. Of this number, dairy cattle work accounts for 3.3, 3.0 devoted to performance testing and 0.3 to program leadership, and poultry work accounts for 3.2, 3.0 devoted to performance testing, and 0.2 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

The effort of the State stations in this area is quite large. It is difficult, however, to make a manpower estimate which would be distinct from that in breeding sections. As the USDA material indicates, much of the effort in DHIA and in poultry testing is cooperative with the States. At several State stations, DHIA records are processed for dairymen on a reimbursable basis. Data derived is used in estimation of genetic parameters, etc., by resident investigators. Similarly, the random sample poultry tests on broiler and egg production strains conducted at State locations provide information on performance and mortality of commercial stocks. Eggs and meat from these poultry tests are used in detailed studies such as strain comparison of chemical constituents and correlation of these items with production traits. -

Animal performance and management records with beef cattle, swine, and sheep are frequently obtained through cooperation with producers, extension service, and industry. In several States, swine testing stations have been established and operated on a self-sufficient basis supported by fees. Research personnel often act in an advisory capacity. In many States, programs of on-the-farm performance and progeny testing of beef cattle have been developed. Again, research and extension people frequently cooperate in this endeavor. At central facilities in some States, bulls belonging to producers are tested for growth and feed efficiency often under the direction of research personnel. Similarly, a limited number of ram testing stations have been developed for indicating growth rate.

No estimate of State station professional man-years is made.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

Poultry

1. National Poultry and Turkey Improvement Plans. During 1963-64, a total of 35.8 million chicken breeding birds in 18.5 thousand flocks and 3.7 million turkey breeding birds in 2.1 thousand flocks were blood-tested and found negative for pullorum and typhoid. These birds produced hatching eggs for 1,979 hatcheries with a hatching capacity of 390 million eggs. In addition to participating in the disease control phase of the Plans, over 38 million chickens and turkeys were in flocks qualified for a breeding classification.

The number of pullorum and typhoid reactors found during the initial blood-testing of breeding stock showed a slight increase over last year. Percentagewise, it rose from .0049 to .0054. With this low rate of infection, it may be expected that these year-to-year variations will occur. With the increasing emphasis on field investigations of all laboratory diagnoses of pullorum and typhoid, it is anticipated that the foci of the infections will be located more quickly and eliminated as potential disseminators of these two diseases.

Although the rate of infection of pullorum and typhoid in breeding flocks has remained at a low level for the past five years, the rate of infection of other paratyphoids has steadily increased. Recognizing this problem, the turkey delegates to the 1964 National Plans Conference voted to include a typhimurium control program in the National Turkey Improvement Plan. These same delegates also recommended a *Mycoplasma gallisepticum* (PPLO) control program as a voluntary part of the Turkey Plan.

For the first time in the history of the National Turkey Improvement Plan, the number of Bronze turkey breeders tested has fallen below 50% of the total number tested.

Reports of exports by Plan participants show that over 92 million units (chicks, poult, and hatching eggs) were shipped to foreign countries during 1963. This represented an increase of 19 million units or 26% over the 1962 exports. A special listing of stocks available for export was published as an aid to the development of international distribution by breeders in the United States. These lists were distributed to prospective purchasers by the United States Agricultural Attache in various countries.

2. Random sample performance tests. The data obtained from random sample production tests have generally been received by the poultry industry as a reliable source of information. When these data are treated by acceptable statistical procedures to minimize non-genetic differences, and then combined by stocks over tests and over years, the information becomes even more reliable as a stock selection guide for commercial poultrymen.

The Poultry Improvement staff collected data from 15 United States and four Canadian random sample egg laying tests in 1963. This information covered the performance of more than 52,000 birds tested in 1,305 pens. Sixteen traits of economic importance were evaluated for the 167 different stocks that were tested.

In 1963, for the first time, the combined analysis was computed from the data obtained over a two-year period, as compared to the single year's data in the past. This procedure permits even more reliable predictions of the performance of the stocks. The analysis took into account the repeatability of stock performance within years and between years as well as the correlation among replicates within a test and within tests over years. The number of tests in which a stock is entered, the environmental or non-genetic differences, and the level of the performance of a stock in relation to other stocks in the same test were also used in the analysis. The results of these computations, expressed as regressed means with 80% confidence limits, are predictions of what the performance of a stock would have been if the stock had been tested at 52 locations over the two-year period.

Performance records made by 3,800 turkeys, representing 34 entries of 23 different stocks, were collected from four random sample turkey meat production tests. The data were analyzed separately by tests, and Duncan's multiple range test was applied to 12 traits to show the statistical significance of differences between entries within a test. The figures were further analyzed by combining the performance of the stocks across tests and computing the regressed mean and LSD range for each of 15 traits for each stock. All turkey test data were published under one cover and distributed to the turkey producers upon request.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Poultry

Breed Distribution of NPIP Participating Flocks by States and Divisions. 1951-52, 1961-62, and 1962-63. ARS 44-2.

Tables on Hatchery and Flock Participation in the National Poultry Improvement Plan by States and Divisions. 1961-62 and 1962-63 and U. S. Summary, 1957-58 to date. ARS 44-3.

Tables on Hatchery and Flock Participation in the National Turkey Improvement Plan by States and Divisions. 1961-62 and 1962-63 and U. S. Summary, 1957-58 to date. ARS 44-4.

Hatcheries and Dealers Participating in the National Poultry Improvement Plan. ARS 44-6.

Annual ROP and Performance Test Summary 1962-63. ARS 44-7.

Participants in the National Turkey Improvement Plan. ARS 44-8.

Turkeys in NTIP Flocks and Their Distribution by States and Varieties, 1951-52, 1961-62, and 1962-63. ARS 44-11.

Turkey Performance Tests. 1963. ARS 44-13.

1963 Report of Egg Production Tests, United States and Canada. ARS 44-79-4.

NPIP and NTIP Official State Agencies. CA-44-7.

Poultry Stocks Available for Export by Breeders or Their Agents in the United States. CA-44-51.

Proposed Changes in The National Poultry Improvement Plan and The National Turkey Improvement Plan.

PRODUCTION INFLUENCES ON POULTRY
Animal Husbandry Research Division, ARS

Problem. Beef, lamb, pork, and poultry are excellent sources of wholesome and digestible animal proteins and fatty acids necessary in maintaining a healthy, appetizing diet. However, these meats must be of high quality, as well as in plentiful supply, if they are to retain their high position and esteem in the minds of consumers. Proper finish, a high proportion of lean, with adequate intramuscular fat, tenderness, full flavor, and color desired by the consumer are the goals the meat producer must strive to attain through breeding, feeding, and management. The quality of cuts and kind of meat are directly reflected in the demand and in the price of the product.

Egg shell strength and yolk quality, strength of wool, fatness, quantity, flavor, color, and tenderness of meat are all known to be influenced by production practices. However, these quality characteristics and many more are not well understood, even though they are of considerable economic importance. Effective measures of evaluating quality differences are of great importance in determining the nature and effect of production practices on the products.

USDA AND COOPERATIVE PROGRAM

This is a continuing program conducted by food product technologists, wool and fiber technologists, biochemists, chemists, physiologists, statisticians, and animal husbandmen engaged in both basic and applied research designed to develop methods and information which will be useful in evaluating quality and quantity of animal products and will be useful in aiding and directing livestock production. Research on beef, veal, lamb, and pork is directed at the influence of selection and breeding, nutrition, physiology, management, and other production variables on carcass and meat quality and quantity. Standards are being applied and adapted for appraisal of slaughter animals, of carcasses, and of meat cuts. The objective of the work with poultry and eggs is to ascertain those factors of nutrition, breeding, and management which contribute to the initial quality of poultry products and their capacity to retain that quality. Studies with wool, fur, and fiber are conducted to determine the physical, chemical, and biological structures and properties of wool and other animal fibers as influenced by production factors. Research on humane slaughter was continued on a reduced scale, primarily to bring to a conclusion some phases of electrical immobilization and physiological responses. The work is conducted at Beltsville, Maryland; Dubois, Idaho; Fort Wingate, New Mexico; and in cooperation with eight State experiment stations. Cooperation is also carried out with the Eastern and Western Utilization Research and Development Divisions, the Human Nutrition Research Division, the Agricultural Engineering Research Division, and the Market Quality Research Division.

The Federal scientific effort devoted to research in this area totals 15.6 professional man-years. Of this number 5.5 are devoted to beef; 1.1 to lamb, mutton, and chevon; 4.0 to pork; 1.0 to poultry and eggs; 2.1 to wool, fur, and fiber; 0.5 to humane slaughter; and 1.4 to program leadership.

A grant with the Polish Academy of Sciences in Poland provides for studies on the color of pork as influenced by heredity, sex, age, feeding, and management. Its duration is for five years (1960-1964) and involves PL 480 funds with \$42,784 equivalent in Polish zlotys.

PROGRAM OF STATE EXPERIMENT STATIONS

Poultry and Eggs. Research on egg quality includes work on the causes and prevention of blood and meat spots and undesirable yolk coloration. Egg shell quality must be good in order to maintain high interior quality in market channels, and the effects of nutrition, heredity, and environment on egg shells are being investigated. The effects of different egg washing techniques on interior quality are also being evaluated. Research is underway on the effect of nutrition and management on chicken and turkey carcass quality traits such as skin pigmentation and a desirable amount of fat.

The total State scientific effort devoted to production influences on animal products research is 51.4 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

Poultry and Eggs. Studies were made of the eggshell weight of eggs with sound and cracked shells. The shells of cracked eggs ranged in weight from 3.5 to 6 grams. Eggs with shells weighing less than 3.5 grams were lost in the laying house due to complete breakage. Eggs having shells weighing more than 6.0 grams were seldom cracked under good handling methods. As the weight of the shell decreased from 6.0 grams to 3.5 grams, the percentage of eggs with cracked shells increased logarithmically, so that the empirical probability of the cracking could be calculated for various shell weights. (AH e4-10)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Poultry and Eggs

McNally, E. H. 1964. The relation of eggshell weight to cracked eggs. Poultry Sci. (Abs.) and (in press) (AH e4-10)

INFECTIOUS AND NON-INFECTIOUS DISEASES OF POULTRY
Animal Disease and Parasite Research Division, ARS

Problem. Annual losses from infectious and non-infectious diseases of poultry, exclusive of parasitisms, are estimated to be at least \$200 million. Continued and expanded basic and applied research are essential to aid in reducing these losses, which inevitably affect cost to the consumer. Added to the initial losses from mortality, reduced weight gains, poor feed utilization, decreased egg production, and lowered quality, are the final losses occasioned by condemnations at dressing plants. United States turkey growers in particular, are faced with a new problem in that a newly discovered infection with a different strain of Mycoplasma is widespread in flocks throughout the country. Resulting condemnation losses at slaughter are often great. The problem is to keep abreast of changing conditions in the field, which present increasingly complex problems requiring basic information.

USDA AND COOPERATIVE PROGRAM

The Department has a long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of poultry. Research is being conducted on the diseases at the following locations.

The Federal scientific effort devoted to research in this area totals 31.3 professional man-years. This effort is applied as follows:

Ornithosis 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Universities of California and Minnesota, and the Agricultural Experiment Stations of Oregon and Texas.

Salmonellosis 3.0 at the National Animal Disease Laboratory, Ames, Iowa, and the Southeast Poultry Research Laboratory, Athens, Georgia.

Pasteurellosis 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Chronic Respiratory Disease Complex 16.7 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the Agricultural Experiment Stations of Connecticut, Delaware, Georgia, Massachusetts, New York, North Carolina, Texas, Virginia, and Wisconsin, and with the University of Minnesota.

Newcastle Disease 4.2 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the University of Maine and the Wisconsin Agricultural Experiment Station, and under a PL 480 Grant to the Institute for Veterinary Research, Pulawy, Poland.

Leukosis 0.3 under cooperative agreement with the Regional Poultry Research Laboratory, USDA, East Lansing, Michigan.

PROGRAM OF STATE EXPERIMENT STATIONS

The major effort by the State stations in this area is being placed on the respiratory disease complex (Airsacculitis) of poultry. Twenty-seven States are cooperating with the Department in three regional projects (NC-65, NE-5, and S-34) on basic and applied aspects of this disease complex. The Department also contributes significantly to this research through cooperative agreements with a number of States. Considerable emphasis is being placed on Chronic Respiratory Disease (CRD). Efforts are being made to improve diagnostic procedures through studies of biological and serological aspects of the causative agent and closely related organisms. The interaction of other infectious agents in causing CRD are being explored. The role of environment in disease outbreaks is being examined and practical methods of treatment and eradication are being developed.

Basic studies are in progress at a number of locations on the structure and composition of Newcastle disease virus. The immunogenic properties of various strains of this agent also are under evaluation with the object of providing improvements on present vaccines and diagnostic materials. The role of nutrition in relation to this disease is being determined. Infectious bronchitis and laryngotracheitis are additional important diseases of the airsacculitis complex being studied. Research is being concentrated on antigenic and immunologic characteristics of these diseases to provide improved preventive methods. Tissue culture modified viruses are being evaluated as improved vaccines.

A number of States are studying the growing problem of Salmonellosis and are tracing the sources of this infection. The effects of nutrition and inter-current diseases on resistance to Salmonella are being determined. Improved methods of diagnosis and control are being developed for ornithosis. Methods of transmission and immunization are being sought for avian leukosis. The causes of diseases such as infectious synovitis, dissecting aneurysm and atherosclerosis are being investigated.

The States are allotting 52.9 professional man-years to poultry disease research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Ornithosis

At the National Animal Disease Laboratory, Ames, Iowa, basic research is in progress on this problem. It is directed toward relationships between psittacosis-group agents found in wild birds and those found in domestic birds and mammals; the differences in their specific antigens, inter-species susceptibility, and other factors which will aid in characterization of the

agents and development of specific diagnostic antigens for the agents.
(Ames, Iowa)

At the University of California, cooperative studies have shown chickens to be susceptible to ornithosis agents of low virulence. The virus was excreted in the saliva and droppings as early as 3 days following intra-tracheal inoculation. The virus could be isolated from organs in the absence of signs of infection for at least 5 weeks, the longest period tested.

The quantity and quality of the ornithosis complement-fixation (CF) antibody component which reacts with the bacterial antigen was found to differ among individuals and animal species following exposure to ornithosis virus. The titers obtained with the Herellea and ornithosis antigens were not always parallel. From a limited number of trials a more sensitive bacterial antigen for detection of common CF antibody was obtained from organisms propagated at room temperature than those grown at 37°C.

Although different batches of the ornithosis yolk sac antigens reacted with anti-ornithosis serums, marked variations in CF sensitivity was observed among them for the same anti-ornithosis serums. By the use of an agglutination test with a formalin inactivated bacterial antigen, antibodies produced by the Herellea bacterium can be detected to differentiate this infection from ornithosis.
(Davis, California)

Continued cooperative studies at the University of Minnesota indicate that the ornithosis virus remains prevalent in the avian population in Minnesota and some adjacent States. One hundred sixteen flocks from Minnesota, Wisconsin and Iowa were tested. Nineteen flocks (16.4%) gave positive reactions by the direct complement fixation test. Ten flocks (8.6%) gave reactions regarded as suspicious. The remaining flocks (75%) were considered negative.

Avian species other than turkeys were also tested serologically and were considered negative.

Parallel complement fixation tests with a bacterial extract antigen and the ornithosis antigen continued to yield a good correlation in results. It would appear feasible to substitute the bacterial antigen for the virus antigen in the presumptive diagnosis of ornithosis in turkeys on a flock basis.
(Minneapolis, Minnesota)

At the Oregon Agricultural Experiment Station, Corvallis, an isolate of the ornithosis agent from a sea gull has been reduced considerably in virulence for turkeys by serial passage through mice (40 passages) and chicken embryos (20 passages). The agent has become more pathogenic for mice and chicken embryos. No evidence of a substantial immunity resulted from injection of the mouse-passaged agent into turkeys. A colony of red mites was established for use in transmission studies of ornithosis.

Twenty percent of a group of turkeys negative to the *Mycoplasma* S6 agglutination test became positive to the test within 7 days after exposure to a dose of virulent ornithosis agent. They became negative again 21 days after the exposure. (Corvallis, Oregon)

At the Texas Agricultural Experiment Station, College Station, turkeys experimentally inoculated with ornithosis and *Pasteurella multocida* (fowl cholera organism) were studied with various diagnostic methods. A combination of stains using the basic dyes and fluorescent techniques were found to be useful in diagnosis. This combination of methods makes it possible to arrive at a rapid positive diagnosis when compared to virus isolation techniques which take at least one week and usually longer. Serology used in conjunction with the staining of the organism with fluorescent stains gives rapid positive identification of this organism.

In a study of a natural outbreak of ornithosis in turkeys, it was evident that the method of spread in the turkey flock was by direct contact. The infection spreads rapidly from turkey to turkey. When clinical signs first appear, introduction into the flock is evidently of recent origin and is probably made by birds of one kind or another.

It is evident from serologic studies made on people working in our laboratory that exposure may produce a slight degree of immunity or antibody titer without clinical signs being observed. (College Station, Texas)(ADP a5-20)

B. Chronic Respiratory Disease Complex

At the National Animal Disease Laboratory, Ames, Iowa, the following work was accomplished:

1. Storage of Mycoplasma Strains. Survival of *Mycoplasma* strains (19 of avian origin, 3 human, 3 canine, and 1 saprophyte) in the lyophilized state and at various storage temperatures was studied. The effect of alternate freezing and thawing was also studied. All strains survived the freeze-drying process and at least 3 or 4 years of storage in the lyophilized state. At -26 C, they survived for at least 10 months, but changes were noted in the colony size and growth rate of cultures stored longer than 10 months. At -65 C, however, there was little loss in viable numbers from 12 months of storage, and no changes in the organisms were apparent. There was considerable variation from strain to strain in resistance to alternate freezing and thawing. Of 16 strains tested, 13 withstood the freezing and thawing better than did the bacterium *Escherichia coli*. At 5 C. storage there was considerable variation in survival between strains. The saprophytic strain, C-15, showed no apparent loss after 9 weeks at 5 C. Most other strains showed rather rapid loss of viable numbers when stored at 5 C. (Ames, Iowa) (ADP a5-21)

At the Southeast Poultry Research Laboratory, Athens, Georgia, the following studies were conducted:

2. Mycoplasma gallisepticum Antigen Production. Sufficient plate antigen was produced during the year to conduct 210,000 tests. In addition to our own research program, the Georgia Poultry Diagnostic Laboratory, Gainesville, was supplied with 150,000 test doses.
 3. Hyperimmunization of Chickens and Rabbits with Mycoplasma gallisepticum (S6, 801 and A5969). The use of small doses of concentrated antigen injected at proper intervals into chickens and rabbits produced antisera with high hemagglutination inhibition titers. It was found that the time of the inoculation is more important in the production of antibodies than the total amount of antigen injected.
 4. Chronic Respiratory Disease Control Program. In a study involving 143,350 broilers, a net savings of 0.347 cents per pound in favor of Mycoplasma-free chicks was demonstrated. Mycoplasma-free broilers cost 11.98 cents per pound to produce versus 12.327 cents a pound for Mycoplasma-positive broilers. Poorest performance occurred where M. gallisepticum-free and commercial birds were mixed in the same houses.
 5. Newcastle disease and Infectious Bronchitis Virus Purification. Examination of field and laboratory strains of Newcastle disease and infectious bronchitis viruses collected for research investigations has shown instances of contamination with PPLO. The possibility is widely recognized that some or all of these strains may also be contaminated with "wild" avian viruses. These contaminants would be unnoticed if they did not cause lesions in chickens or in the chick embryo. They also cause misleading results or interpretation of serological experiments and may account for varying results in different laboratories. The contaminants are being eliminated by filtration and limit dilution techniques. (Athens, Georgia) (ADP a5-17, a5-18, and a5-23)
- Cooperative studies at the Connecticut Agricultural Experiment Station, Storrs, were conducted on several phases of the problem, viz:
6. Serology. Production of Mycoplasma gallisepticum antigen for chronic respiratory disease (CRD) testing was continued under AIQ special license number 237. Antigen was shipped to 25 States and 9 foreign countries. Approximately 700,000 doses were produced this past year, a 35% increase over the previous year. A CRD testing program on a flock or partial sample basis was made available to the poultry farmers of Connecticut.
 7. Control. a) Immunization of 8-12 week old birds with living pathogenic M. gallisepticum was conducted with the goal of producing progeny free of this Mycoplasma. Records of the performance of vaccinated birds were in good agreement. Vaccinated birds performed as well as non-vaccinated birds in regard to growth rate, hatchability, fertility, egg production, and

mortality. In addition, vaccination of birds in one house which was a problem in 1961-1962 resulted in a 10% increase in egg production in 1963. Embryo transmission of M. gallisepticum from birds vaccinated at a young age appear to be negligible and the feasibility of producing M. gallisepticum-free birds by this procedure has been demonstrated in at least one case. b) Killed vaccine: Attempts at vaccination against M. gallisepticum with killed vaccine have been rather unsuccessful. The possibility of vaccinating with PPLO killed by gamma irradiation at a level which does not denature protein was explored. c) Isolation: Using a filtration method (Millipore 0.45 micron filters), successful isolations of M. gallisepticum from the trachea of 7 groups of birds were made in which no isolations were made due to bacterial growth in a similar medium containing thallous acetate and penicillin but not filtered. d) M. gallisepticum antigen: The difficulties of growing M. gallisepticum in good yields for antigen production are well known to all working in this area. To overcome these difficulties the research at this laboratory has been in two directions - to provide a more optional medium, and to devise methods for more efficient use of the antigen produced by current methods. (Storrs, Conn.)

Cooperative research at the Delaware Experiment Station, Dover, has been concerned primarily with the investigation of the value of various antibiotics (Aureomycin, Terramycin, Erythromycin and Tylosin) for the control of M. gallisepticum infection in experimentally infected broiler chickens. Tylosin was the most effective antibiotic for the control of M. gallisepticum infection under the conditions of these experiments. While this drug did not completely suppress the infection, it was more effective than the other drugs tested. Aureomycin and Terramycin gave some control of the infection while Erythromycin was the least effective drug employed. (Dover, Delaware)

At the Georgia Agricultural Experiment Station, Athens, cooperative studies showed a) broiler flocks on non-medicated feed did as well as those on medicated feed: b) the success or failure of vaccination programs against respiratory virus diseases depends mainly on the degree of latent Mycoplasma infection: c) CRD-free broilers grow heavier and have fewer condemnations than PPLO-infected birds. Birds from the same breeder flock sources have lower weights and higher condemnations when grown in a controlled environmental house even during the winter months. This was probably due to easier spread of infection through the air in a house with forced draft ventilation. (Athens, Georgia)

At the Massachusetts Agricultural Experiment Station, Amherst, cooperative studies and the results were:

8. Transmission. Transmission of CRD through cohabitation of CRD-serologically positive birds and susceptible birds may occur, under certain conditions, only after a prolonged exposure period. Fecal material of CRD serologically positive birds fed to susceptible birds yielded transmission of the disease.

9. Serology and immunity. a) Parental agglutinin studies. Varying degrees of agglutination of M. gallisepticum (S6) antigen may be produced by sera from day-old chicks obtained from positive dams. Individual dams may produce chicks of similar serologic status, but there may also be considerable variation among the chicks within the same hatch as well as between hatches. Tylosin therapy of the serologically positive dam does not appear to alter the serologic reaction of the dam or its progeny. b) Comparison of serologic tests. Serologic tests (rapid-serum-plate, tube agglutination, and hemagglutination-inhibition) on samples collected from known negative birds showed close agreement. This also appears to be true for known strongly positive birds. However, the results of the different testing methods on samples from some positive birds are not in agreement, even though the results of successive tests on the same positive birds appear to be highly reproducible for the respective methods.

10. Response of CRD to medication. Tylosin, given in adequate dosage, may be highly effective in the control of experimental CRD infection. Eradication of CRD from infected breeding stock may be accomplished through sound management and tylosin medication of dams and their progeny.

11. Control and eradication. CRD-free stock can be produced, maintained, and reproduced if adequate sanitation and preventive practices are adopted. The majority of negative premises continue to remain negative on successive years. Significant progress has been made in establishing CRD-free stock and this stock will become available in larger numbers to the industry in various parts of the country. (Amherst, Massachusetts)

At the University of Minnesota, Minneapolis, the following cooperative studies have been conducted:

A whole blood plate antigen is being used as a screening test for the detection of Mycoplasma gallisepticum infection in chickens. Two experiments are being conducted in an effort to produce M. gallisepticum-free chickens. The pathogenicity of 19 avian Mycoplasma serotypes was determined. The two primary pathogens are the A serotype (S₆ strain) and the H serotype (N strain). The S serotype (infectious synovitis strain) is capable of producing synovitis but not airsacculitis. A few of the other serotypes produce a local lymphofollicular reaction while still other serotypes appear to be non-pathogenic.

Attempts have been made to establish "Mycoplasma free" turkey poults by the use of tylosin. A significant reduction in the incidence of airsacculitis in the day-old poult was seen following the dipping of the egg in a solution containing 1000 ppm of tylosin. This treatment also resulted in a reduced condemnation from airsacculitis when these birds were marketed at 16-24 weeks of age. The H serotype (N strain) appears to be the primary cause of the airsacculitis seen in the day-old poults.

A satisfactory media has been developed for the growth of the H serotypes of Avian Mycoplasma. This has made it possible to develop a serum plate antigen for the detection of this serotype in Minnesota turkeys. A study of the incidence of this serotype is being planned for the coming year.

(ADP a5-17)

Field investigations were conducted on clinical outbreaks of infectious sinusitis on eleven premises during the past year. While two investigations are still pending, none of the outbreaks have been traced back to a Minnesota Mycoplasma gallisepticum tested breeder flock.

Studies were continued on the effect of environmental conditions on the airsacculitis syndrome in turkeys. Two experiments were conducted in an effort to raise two "Mycoplasma free" fryer roaster turkey flocks. The eggs were dipped in both experiments, in the second experiment the poults received a 5-day treatment of tylosin in the drinking water. The airsacculitis seen both at one day of age and at the time of processing was minimal in both flocks. Mycoplasma could be isolated from the egg dipped flock but not from the flock where the eggs were dipped and the poults were treated.

Air samples were taken in the environmental turkey buildings throughout the last two experiments. The count rose to a high level during the first three to five weeks of the experiment and remained high throughout the remaining 10 to 14 weeks. The significance of this data is unknown at the present time.

(Minneapolis, Minnesota) (ADP a5-21)

Cooperative studies at the New York State Agricultural Experiment Station, Ithaca, have given the following results:

Mycoplasma gallisepticum was destroyed in eggs when they were dipped in 1500 ppm solution of Spiramycin. Tylosin in concentrations of 1000 ppm has continued to be effective as a dip for infected eggs. Eggs from three infected breeding flocks, dipped in Tylosin solution (eggs at 37C, dip solution 5C, dip time 5 minutes) resulted in 20 clean hatches and one infected hatch. The single failure was due to inadequate warming of the eggs prior to dipping. Of the 31 hatches from the same flocks that were not dipped, 5 were clean and 26 infected. One clean breeding flock (itself derived from dipped eggs) which produced 5 clean hatches from undipped eggs became infected in an unknown manner. In general, dipping of infected eggs in an antibiotic solution promises to be a quick and effective way to produce clean progeny from infected dams.

Immunization of chickens with live cultures to prevent egg transmission of PPLO has two inherent difficulties - a) the delay between immunization and egg production, and b) the risk of inducing complicated CRD when a virulent immunizing culture is used. Non-pathogenic immunizing cultures do not produce solid immunity.

The isolation of M. gallisepticum from tracheal swabs, does not necessarily mean that such birds are spreaders. On the other hand, spread of infection occurred from birds that did not yield PPLO from tracheas. Positive agglutination tests appeared later than culture isolations at the outset of infection. A high incidence of positive serological tests persisted for a much longer time than it was possible to make an equivalent number of PPLO isolations from the trachea. One must consider any bird with a positive agglutination test as a potential spreader. (Ithaca, New York)

Cooperative studies at the North Carolina Agricultural Experiment Station, Raleigh, determined the microscopic changes taking place in the turkey with experimentally produced sinusitis. The reaction in the sinus wall was characterized by an increased blood supply, a progressive invasion by white blood cells, the collection of fluids in the tissues, and the formation of an exudate in the sinus cavity. This study adds to the knowledge which may be used in the diagnosis of natural and experimental sinusitis of turkeys. (Raleigh, North Carolina)

At the Texas Agricultural Experiment Station, College Station, the following cooperative studies were reported:

Chronic Respiratory Disease Eradication. Preliminary cooperative attempts to bring 20,000 replacement chickens, hatched from M. gallisepticum free breeding stock, into production free of M. gallisepticum failed. Efforts are being continued to develop commercial breeding operations free of M. gallisepticum.

M. gallisepticum Antigens: Cooperative studies have been made with the ADE Division of the USDA to establish standard M. gallisepticum production and testing protocols. USDA experimental lots of antigen have been subjected to extensive evaluation. Lots made with the Adler S-6 strain have not been entirely satisfactory due to granularity and hypersensitivity. Lots of antigen made with the Massachusetts A5969 strain have been quite reliable. Both antigens are more sensitive than antigen made with the Iowa 801 strain. USDA H.A. antigen (Bio-20) fails to detect low titering M. gallisepticum reactors when used according to standard protocol.

Non-Specific Reactions to the M. gallisepticum Plate Test. Preliminary studies demonstrate that turkeys immunized with erysipelas bacterin transitory reactions to the M. gallisepticum plate test. Reactions appeared as early as 7 days post-inoculation and persisted as long as 22 days post-inoculation. The reaction and persistence of reaction levels were higher with USDA A5969 antigen than with TAES 801 antigen.

Turkeys immunized with fowl cholera bacterins apparently do not develop significant transitory reactions to the M. gallisepticum plate test. (College Station, Texas)

In cooperative studies at the Virginia Agricultural Experiment Station, Blacksburg, the following results were reported:

The ultrastructure of 17 strains (15 species) of Mycoplasma was studied with the aid of the electron microscope. The average diameter of mature cells, elementary bodies and ribosomes, and the thickness of the 3 layers of cell membranes was determined. The presence or absence of developing elementary bodies, internal membranes (mesosomes) and vacuoles was determined, and the nature of special features was described.

The morphology and ultrastructure of 2 strains of Mycoplasma gallisepticum was studied with the aid of the electron microscope. Evidence supporting the viewpoint that M. gallisepticum multiplies by the formation of elementary bodies was obtained.

A severe Mycoplasmal salpingitis was produced in turkeys. In chickens, the caseous plug associated with Mycoplasmal salpingitis was found either to be resorbed or to be passed from the vent before the first eggs were laid. In addition, many eggs had little or no mineral deposition on the shell membrane.

"Mycoplasma free" flocks have been maintained at this Station for seven years by appropriate management procedures. (Blacksburg, Virginia)

At the Wisconsin Agricultural Experiment Station, Madison, cooperative studies show that of the variables in the environment of confined turkeys that have been measured, three (temperature, carbon dioxide, and ammonia) are being studied in environmental chambers. Ammonia at the measured level is damaging to the respiratory tract of chickens and turkeys. After prolonged exposure, changes in the tracheal epithelium can be demonstrated histologically. Even after short exposure the birds are markedly more susceptible to Newcastle disease. On the other hand, carbon dioxide at levels that exist in commercial establishments has rendered the birds less susceptible to Newcastle disease.

The only Mycoplasma found in the Charmany study flocks is N strain (H serotype). Primary attention has been given to improved methods for the culture of this strain and to improved methods for its serological detection. The presence of the organism has been followed from the laying flock, to the hatchery, and in the hatched poults from one day of age, by means of a weekly sampling, to the 14-week market age. The condemnation status of each flock is determined by a Federal inspector at an approved plant. The presence of N strain and the extent of respiratory disease in market age birds is being carefully assessed.

(Blacksburg, Virginia) (ADP a5-21)

C. Salmonellosis

At the Southeast Poultry Research Laboratory, Athens, Georgia, work on this problem has shown the following results:

1. Salmonella typhimurium Stained Antigen. Further refinements have been made in a stained antigen to detect carriers of Salmonella typhimurium infection in poultry flocks by either the rapid whole-blood or serum plate test. A single culture of S. typhimurium has been selected for preparation of the antigen and several changes have been established in its processing with resulting improvements in its sensitivity and quality.

2. Salmonella Penetration through Shells of Fresh Chicken Eggs. In an experiment designed to study the penetration of Salmonella organisms through the shells of fresh chicken eggs, it was established that one or more eggs in all groups studied were penetrated by the Salmonella at the end of 24 hours, the time of earliest sampling. All eggs used in these studies had been penetrated by the organisms at the end of the sixth day.

(Athens, Georgia) (ADP a5-2(Rev.))

D. Pasteurellosis

At the National Animal Disease Laboratory, Ames, Iowa, studies have been conducted on 1) dissociation, 2) histopathology, 3) reservoirs of infection, and 4) characterization of antigens.

1. Dissociation. The genetic loss of ability of an avian strain of P. multocida to form a capsule resulted in a marked loss of virulence but not of immunogenic antigen(s) which was easily extracted with saline. There was also a direct relationship of virulence to colonial morphology.

A highly virulent culture from an acute case of fowl cholera produced fluorescent colonies which mutated in vitro and produced blue colonies. Organisms from the blue colonies also mutated and produced gray colonies. Organisms from blue colonies reverted in vivo and produced fluorescent colonies. Organisms from the fluorescent colonies occurred singularly or in pairs, were capsulated and virulent for chickens, turkeys, rabbits, and mice when administered via the mucous membranes of the upper air passages. Organisms from the blue colonies occurred singularly or in pairs, were non-capsulated and avirulent for chickens and mice, but virulent for rabbits and slightly virulent for turkeys. Organisms from gray colonies were non-capsulated, avirulent, and grew as a tangled mass of filaments. Organisms from the three types of colonies could not be differentiated by biochemical test or by serologic and immunologic response in chickens.

2. The Histopathology of Acute Fowl Cholera in Mature Chickens. Tissues from chickens that died from acute fowl cholera were compared microscopically with tissues from uninfected chickens to determine the histopathologic changes produced, and to characterize the type of tissue response in order to gain

insight on the cause of death. Lesions in the infected tissues were - generalized passive hyperemia; heterophilic infiltration of the lung, liver, adrenal, kidney, and thyroid; heterophilic depletion and hemopoietic cell degeneration in the bone marrow; generalized bacteremia, and acute focal necrotic hepatitis. Generalized passive hyperemia, the most pronounced and significant lesion observed, resulted from cardiac insufficiency, atony of veins and capillaries, or both, and is indicative of shock. The acute hemorrhagic enteritis commonly described in fowl cholera was shown to be a very severe acute passive hyperemia rather than an inflammatory reaction.

3. Fowl Cholera: Susceptibility of various animals and Their Potential as Disseminators of the Disease. Various animals were exposed to a culture of Pasteurella multocida isolated from a chicken dead of acute fowl cholera to determine their susceptibility to fowl cholera and their potential as carriers of the organism. Pigeons, sparrows, mice and rabbits died of acute septicemia when exposed intranasally. Rats, ferrets, guinea pigs, a sheep, a pig, and a calf failed to elicit any noticeable response to an intranasal exposure. There was a transitory temperature rise in a 7-week-old mink. The organisms were reisolated from the nasal passages of the calf and the pig 34 days after exposure and were still highly virulent for chickens. For this reason, other domestic farm animals may prove to be potential carriers of this disease.

One of 5 rats, 1 of 2 mink, and 11 of 19 mice fed viscera of chickens dead of fowl cholera developed a nasal infection, pneumonia and fatal septicemia, respectively. Since most of the animals tested were susceptible to or potential carriers of the fowl cholera organism, these experiments re-emphasized the necessity of a good sanitary program in the control of fowl cholera. The practice of feeding chickens dead of fowl cholera to other animals, or allowing domestic animals, free-flying birds or rodents to come in contact with dead or diseased birds, leads to a source of infection for neighboring or replacement flocks.

4. Isolation and Preliminary Characterization of an Antigen from Pasteurella Multocida which can induce immunity in chickens. It is generally recognized that whole cell preparations of the fluorescent mutant of Pasteurella multocida effectively immunize chickens against the same strain. It has been found that an immunizing agent can be consistently extracted with cold saline from the formalinized cells of the highly virulent fluorescent form, the relatively avirulent blue form, or a new avirulent chain-growing grey form. An immunogenic polysaccharide remains in the saline solution after extraction with an equal weight of phenol. It gave an absorption curve typical of a hexose in the phenol-sulfuric acid reaction and showed 0.8%N by the Kjeldahl method. Seven chickens were injected subcutaneously with the polysaccharide emulsified with Bayol F-Arlacel A, and challenged 19 days later with the virulent strain. All seven chickens were protected, whereas all seven controls died. (Ames, Iowa) (ADP a7-25)

Under a PL 480 Grant at the Veterinary Research Institute, Pulawy, Poland, research showed that high temperatures accelerate the mortality of chickens infected with fowl cholera. Tranquilizers seem to slow the rate of mortality in cholera-infected chickens. (E21-ADP-7)

E. Bluecomb Disease

In cooperative studies at the University of Minnesota, Minneapolis, an enterovirus and a bacteria of the genus *Vibrio* have been found quite consistently in the intestinal tracts of turkeys suspected of having bluecomb disease. In control turkeys, the enterovirus has not been isolated. However, an occasional *Vibrio* has been found. Pure cultures of either of these organisms alone, or mixtures of the two, when reinoculated into turkey poults, produced no observable disease. Rapid serial poult passage did not increase pathogenicity and protective immunity to bluecomb disease did not develop when such cultures were fed over a 4-week period.

Large numbers of *Vibrio* have been found in turkey poults showing bluecomb disease after being inoculated with bluecomb seed filtrates (miliopore .22u) from which *Vibrio* could not be observed nor isolated. *Vibrio* have been found in the water from troughs watering infected turkey flocks, and have been found in chickens showing no symptoms of bluecomb disease. *Vibrio* isolated from swine with diarrhea caused no symptoms of bluecomb disease when inoculated into turkey poults.

Germ free studies and blood analysis, as well as other studies, are continuing toward determination of the possible relationship that *Vibrio* or the enterovirus may have with bluecomb disease of turkeys. (Minneapolis, Minn.) (ADP a5-19(C))

F. Newcastle disease

Studies at the National Animal Disease Laboratory, Ames, Iowa, showed that the immune response induced in chickens with two doses of a beta-propiolactone-killed Newcastle disease vaccine, administered subcutaneously at the second, and again at the twelfth week of age, prevented mortality, paralysis, and decrease in egg production after challenge with a virulent strain of Newcastle disease virus inoculated by either the intratracheal or intramuscular route. In unvaccinated birds, the intramuscular route of challenge produced greater mortality than the intratracheal route.

The virus neutralization capacity of serums of vaccinated birds correlated with the immune status of the birds throughout the experiment, for vaccinated birds had significantly high serum neutralization titers up to 70 weeks of age. The HI titers of vaccinated birds remained significantly high for only 50 weeks. Isolation of Newcastle disease virus 48 hours after challenge as an indication of the immune state of the bird was of no significance, since the virus was isolated from both vaccinated and unvaccinated birds after challenge. (Ames, Iowa)

Through cooperative research at the University of Maine, Orono, a Specific Pathogen Free (SPF) program has been conducted on broiler and breeder flocks during the past year, 1963-1964. A rigid set of standards for isolation and husbandry are required to conform to the program. A total of 23 SPF breeding flocks, comprising 143,337 birds have been on the program and have been found free of S_6 PPLO. Altogether 273,845 birds were tested for S_6 PPLO but many of these were not on the SPF program, and 5,845 samples were positive.

The program included a total of 68 SPF broiler flocks, totaling 1,324,648 birds which were followed from day-old chicks to processing in the plant. It was found that PPLO clean chicks, when placed on a clean farm, never became infected during the broiler-growing period of 9 weeks. Only killed Newcastle disease vaccine is used in this program. (Orono, Maine)

Cooperative research at the Wisconsin Agricultural Experiment Station, Madison, was directed toward -

1. Fundamental studies of Newcastle disease virus. With rare exceptions, all Newcastle disease virus (NDV) isolates that have been subjected to plaquing have been found to contain a mixed population consisting of 2 or more plaque types. It has been learned that the 2 plaque lines (S and L) derived from a single strain (Herts) differ in adsorption pattern, growth rate, virulence for chickens and antigenicity. Since these are most important properties, it is necessary to plaque purify all isolates before further characterizing them. Specific antisera must be prepared to the derived plaque lines rather than to the heterogenous wild strains. Two methods for calculating relatedness of antigens are being compared to determine the best procedure to use with Newcastle disease virus strains.

With the procedures based on plaque lines, it may now be possible to establish meaningful antigenic and pathogenic groups for epizootiological studies. In anticipation of such studies, the growth of NDV at aberrant temperatures and in cells of a wide range of species is being investigated.

2. Maintenance of the repository of strains of Newcastle disease virus. The study center for Newcastle disease virus was housed June 1, 1964, in a special laboratory in the new Veterinary Science Building. When the new lyophilizer arrives, the laboratory will be fully equipped. New strains have been added during the year; old stocks have been replenished and records have been up-dated.

3. International Symposium on Newcastle Disease. The proceedings of the International Symposium on Newcastle Disease as an Evolving Pathogen is now in proof. Copies are expected from the University of Wisconsin Press in late September.
(Madison, Wisconsin) (ADP a5-18)

Research at the Veterinary Research Institute, Pulawy, Poland, under a PL 480 Grant, shows no change in the pathogenicity of Newcastle disease virus after 15 passages through 3-week-old chickens with artificially induced hypovitaminosis B₂ and B₁₂. (E21-ADP-2)

G. Avian Leukosis

Results of cooperative research on this problem at the Regional Poultry Research Laboratory, USDA, East Lansing, Michigan, will be reported by the Poultry Research Branch of the Animal Husbandry Research Division. (ADP a5-22)

H. Fowl Plague

Under a PL 480 Grant at the Instituto Jamie Ferran de Microbiologia, Madrid, Spain, research was conducted on fowl plague virus cultivated in cell cultures of chick embryos. The fowl plague virus cultivated in cultures of chicken embryo cells was inactivated with heat and combined with oil adjuvants prior to use for the immunization of chickens. Vaccines prepared in this manner seem to protect large numbers of animals from challenge with infective virus. (Madrid, Spain) (E25-ADP-1)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chronic Respiratory Disease Complex

- Alls, A. A., Cover, M. S., Benton, W. J., and Krauss, W. C. 1964. Treatment of Hatching Eggs for Disease Prevention-Factors Affecting Permeability and a Visual Detection of Drug Absorption. Avian Dis., VIII(2):245-256.
- Andersen, D. P., Cherms, F. L., and Hanson, R. P. 1964. Studies on measuring the environment of turkeys raised in confinement. Poult. Sci., 43: 305-318.
- Chandiramani, N. K. 1963. Viability of Mycoplasma gallisepticum under different environmental conditions. Thesis for M.S. degree from University of Massachusetts.
- Chute, H. L., Stauffer, D. R., Cuzzo, R. F., and O'Meara, D.C. 1964. Progress in PPLO Eradication in Chickens. Maine Farm Research, July.
- Chute, H. L. 1964. The Future of CRD Control. Maine Poultry Improvement Association Yearbook.
- Fabricant, J., and Levine, P. P. 1963. Infection in Young Chickens for the Prevention of Egg Transmission of Mycoplasma gallisepticum in Breeders. Proc. 17th World Vet. Congress:1469-1474.
- Gross, W. B. 1963. Pathology of the B₁ strain of Newcastle disease in chickens. Avian Diseases, 7:417-422.
- Grumbles, L. C., Hall, C. F., and Cummings, Georgia. 1964. Characteristics of Avian Mycoplasma (PPLO) in Tissue Cultures of Human and Avian Cells. Avian Diseases, 8:274-280.
- Kelton, W. H., and Van Roekel, H. 1963. Serological studies of Mycoplasma (PPLO) of avian origin. Avian Diseases, 7:272-286.
- Kleckner, A. L., Hall, C. F., and Fabricant, J. 1963. "Mycoplasma" in Methods for Examination of Poultry Biologics. Rev. Ed. National Academy of Science - Natl. Res. Council Publ. 1038.
- Morowitz, H. J., Tourtellotte, M.E., and Pollack, M. E. 1963. Use of porous cellulose ester membranes in the primary isolation and size determinations of pleuropneumonia-like organisms. J. Bacteriol. 85:134-136.
- Olesiuk, O. M., Van Roekel, H., and Chandiramani, N. K. 1964. Antibiotic medication of chickens experimentally infected with Mycoplasma gallisepticum and Escherichia coli. Avian Dis., 8:135-152.

Smith, S. L., Van Demark, P. J., and Fabricant, J. 1963. Respiratory Pathways in the Mycoplasma I. Lactate Oxidation by Mycoplasma gallisepticum. Jour. Bacteriol. 5:893-897.

Solana, P. 1964. The influence of certain environmental factors on disease transmission in turkeys. Thesis for M.S. degree from the University of Wisconsin.

Tourtellotte, M.E., Jensen, R. G., Gander, G. W., and Morowitz, H. J. 1963. Lipid composition and synthesis in the pleuropneumonia-like organism, Mycoplasma gallisepticum. J. Bacteriol. 86: 370-379.

Tourtellotte, M. E. 1963. The isolation of Mycoplasma from grossly contaminated material by use of membrane filters. Proc. 35th N.E. Conf. on Avian Diseases, Univ. of Massachusetts, Amherst, June 17-19.

Pasteurellosis

Heddleston, K. L., and Watko, L. P. 1963. Fowl Cholera: Susceptibility of Various Animals and Their Potential as Disseminators of the Disease. Proc. 67th Ann. Meet. USLSA.

Rebers, P. A., and Heddleston, K. L. 1964. Isolation and Preliminary Characterization of an Antigen from Pasteurella multocida which can induce Immunity in Chickens. Fed. Proc., 23(2):142.

Newcastle Disease

Gill, Eugene and Stone, H. D. 1964. Newcastle Disease: Immune Response in Chickens to a Beta-Propiolactone-Killed Virus Vaccine. Avian Diseases, VIII: 62-71.

Ornithosis

Pierce, K. R., Moore, R. W., and Bridges, C. H. 1963. Experimental Ornithosis in Ewes. Amer. J. Vet. Res., 24(103):1176-1188.

Pierce, K. R., Moore, R. W., and Carroll, L. H. 1964. Experimental Transmission of Ornithosis from Sheep to Turkeys. Amer. J. Vet. Res., 25(107)

Shimizu, Y., and Bankowski, R. A. 1963. The nature of the cross reactions between a bacterium of the genus Herellea and Ornithosis virus in complement fixation. Amer. J. Vet. Res., 24:1283-1290.

Shimizu, Y., and Bankowski, R. A. 1963. The characteristic of a bacterium used in complement fixation tests for ornithosis. Avian Dis., 7:331-341.

Salmonella

Grundboeck, M. 1963. Pathophysiology of the Pituitary-Adrenal Axis in Birds. Polskie Archiwum Weterynaryjne, 8(3) (PL 480)

PARASITES AND PARASITIC DISEASES OF POULTRY
Animal Disease and Parasite Research Division, ARS

Problem. Parasites and parasitic diseases probably cost the poultry industry many millions of dollars annually by causing intestinal disturbances, emaciation, retarded growth, reduced egg production, and deaths. Parasites are ubiquitous, many times insidious, and often overlooked until birds are damaged irreparably. Early diagnosis is difficult, and reliable treatments for many devastating parasitoses are not available. Moreover, some management practices, intended to avoid spread of parasites and to control them, have been found ineffectual as is shown by the increasing importance of certain parasites in broiler production. The problem is to develop, through a planned, balanced program of basic and applied research, methods for preventing, controlling or eradicating parasitic diseases, thus affording economical production of healthy poultry and sound products in supplies adequate to meet the needs of an expanding population.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving parasitologists, biologists, and chemists, engaged in both basic studies and the application of known principles to the solution of the problem of parasites and parasitic diseases of poultry.

The Federal scientific effort devoted to research in this area totals 5.5 professional man-years. This effort is applied as follows:

Control of Coccidiosis 2.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Biology of Nematode Parasites 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Biological investigations of protozoan parasites and parasitic diseases, with special reference to those of the gastrointestinal tract 2.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

PROGRAM OF STATE EXPERIMENT STATIONS

The principal effort being placed in this area by the States is on coccidiosis. Factors influencing immunity to this disease are being determined and prevention of outbreaks by means of vaccination is being evaluated. The role of nutrient elements in affecting severity of the condition is under study. Several States have work in progress on the blackhead parasite of poultry to learn methods of building resistance against this organism. Other research is in progress to determine life cycles of poultry tapeworms and capallarids and to determine the effects of these parasites on egg production, weight gains and feed efficiency.

There are a total of 2.0 professional man-years allocated to research on parasites of poultry at the States.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Biology of Nematode Parasites

Research at the Beltsville Parasitological Laboratory revealed that a mash containing 0.5 percent thiabendazole removed all gapeworms, Syngamus trachea, from 97 percent of 67 turkey poults that were given the medicated feed for 9 to 20 days. In the aggregate, the regimen removed 96 percent of the total worm populations in the treated birds and was well tolerated in all respects. Systemic action of the drug was indicated by its anthelmintic effect against worms that had migrated from the intestine to the trachea before treatment was started. (Beltsville, Maryland) (ADP b4-10)

B. Biological Investigations of Protozoan Parasites

At the Beltsville Parasitological Laboratory the following work was conducted: As evidenced by oocyst output and extent of lesions following experimental inoculation of similar doses of oocysts, 1-day-old chicks were less susceptible to infection with the coccidium, Eimeria acervulina, than were 3-day-old chicks. This is considered to be due, at least in part, to the inability of the gizzards of 1-day-old chicks to break the walls of the oocysts which are tough and more-or-less impervious. In chickens 3 - 44 days of age, the oocysts were broken in the gizzard and the liberated sporocysts passed on into the intestine where the sporozoites were activated to escape from the sporocysts. The gizzards of 1-day-old chicks have poorly developed musculature and smooth inner linings, whereas the gizzards of 3-day-old chicks are relatively muscular and are lined with a rough material (koilin). Examinations of gizzard and intestinal contents of chicks previously inoculated with massive doses of oocysts showed that much higher percentages of sporocysts were liberated in the gizzards and much higher percentages of sporozoites excysted in the small intestines of 3-day-old chicks than in 1-day-old chicks.

Three species of earthworms have been found to be true, but not obligatory, vectors in the transmission of Heterakis gallinarum, the cecal worm of chickens, turkeys, and ring-necked pheasants, and in the transmission of the protozoan, Histomonas meleagridis, the causal agent of blackhead.

The pancreatic enzymes necessary for excystation of Eimeria acervulina, and perhaps all other poultry coccidia, are trypsin and chymotrypsin. In the presence of a bile salt, these enzymes digest the protein-like sporocystic plug and allow the sporozoite to excyst. Lipase and carboxypeptidase, the other pancreatic enzymes, are without effect. (Beltsville, Maryland)
ADP b4-11)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Protozoology

Farr, Marion M., and Doran, David J. 1963. Survival of Eimeria acervulina oocysts in suspensions in the laboratory. Virginia J. Sci., 14:165.

Lund, Everett, and Farr, Marion M. 1964. Protozoa. Chapter in DISEASES of POULTRY, by Biester and Schwarte. Iowa State University Press, Ames.

Lund, Everett E. 1963. Histomonas wenrichi n. sp. (Mastigophora: Mastigamoebidae), a nonpathogenic parasite of gallinaceous birds. J. Protozool. 10:4:401-404.

Lund, Everett E., Wehr, Everett E., and Ellis, David J. 1963. Role of earthworms in transmission of Heterakis and Histomonas to turkeys and chickens. J. Parasitol., 49(5-Sec. 2):50.

Helminthology

Wehr, Everett E., and Hwang, J. C. 1964. The life cycle and morphology of Ascaridia columbae (Gemelin, 1790) Travassos, 1913 (Nematoda:Ascarididae) in the domestic pigeon (Columba livia domestica). J. Parasitol. 50:131-7.

POULTRY INSECTS

Entomology Research Division, ARS

Problem. Numerous species of insects, mites, and ticks are common pests of poultry throughout the country and if not controlled can make poultry raising unprofitable. They cause poultry to look unsightly, reduce weight gains and egg production, and mar the skin, which results in downgrading of quality and lower market prices. Pests such as black flies and mosquitoes transmit leucocytozoon and fowl pox disease which exact a heavy toll in death and unthrifty poultry each year. House flies spread parasites and enteric diseases which may decimate flocks. Safer, more effective non-residue-forming insecticides are needed to combat these poultry pests and vectors of diseases of poultry. Better materials are needed for direct application to poultry or in poultry houses to control lice, mites, and ticks and for use as larvicides or fly baits to control flies. Materials are especially needed which, when given in feed or water, would act systemically to control external pests and render droppings toxic to fly larvae. Exploratory studies are needed to investigate possibilities of developing attractants, chemosterilants, antimetabolites, or other new methods of combating poultry pests. Biological and sanitation methods of control offer excellent possibilities for control and need to be emphasized. There is a special need to investigate the roles of insects, ticks, and mites in the transmission of poultry diseases.

USDA AND COOPERATIVE PROGRAM

A continuing study is underway in the Department involving basic and applied research on insects, mites, and ticks that affect the health and productivity of poultry. Studies are designed to determine breeding habits and reproductive capacities of various poultry pests and to gain further knowledge on the nature of resistance of these pests to certain insecticides. Work at present is devoted mostly to lice and the northern fowl mite, and to the house fly, which breeds abundantly in poultry droppings. A newly expanded program aims to find new ways to control pests of poultry with special emphasis on use of chemosterilants, antimetabolites, attractants, and non-insecticidal materials and methods. Current studies in this field are largely limited to house flies. They include investigations of physical and mechanical methods for controlling house flies being conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions.

Research is concerned with the development of more effective insecticides for the control of poultry pests. New chemicals are screened in the laboratory for contact and residual toxicity to lice and mites attacking poultry and to house flies, and promising ones are tested for effectiveness under practical field conditions. New methods of utilizing insecticides more efficiently and safely are being investigated, with special attention to finding materials that, when given orally in water or feed, will act systemically to kill lice and mites on the poultry, and render the droppings toxic

to fly larvae. Efforts are also being given to methods of sanitation and management to control breeding in accumulations of manure in poultry houses. Studies are conducted to determine the occurrences of residues in tissues of poultry treated with insecticides. Work is done in cooperation with State Experiment Stations and poultry raisers at Gainesville, Fla., Stoneville, Miss., Corvallis, Oreg., and Kerrville, Tex. Additional research is conducted at Lake Charles, La.

The Federal scientific effort devoted to research in this area totals 2.2 professional man-years. Of this number, 0.4 is devoted to basic biology, physiology, and nutrition; 0.9 to insecticidal and sanitation control; 0.2 to insecticide residue determination; 0.6 to insect sterility, attractants and other new approaches to control; and 0.1 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

The States are performing both basic and applied research on poultry insects. Studies are in progress to determine the distribution and amount of damage caused by the more than 50 external parasites of poultry in the various States. As the extent of injury is determined, harmful species are studied to obtain information on their life histories and ecology. For example, in the northern fowl mite, the rate of population increase, the incubation periods of the eggs and duration of the immature stages are being studied. The effects of various population levels in production and fertility of eggs are also under investigation. The development of laboratory rearing methods is a pre-requisite for much of this research.

Control studies include comparisons of insecticides. New materials are constantly being evaluated as replacements for chemicals to which some poultry pests have been resistant. Treatment methods range from feed additives, which prevent fly development in manure or provide systemic control of parasites on the birds to direct or area applications. Insecticide residue analyses are performed to determine concentrations of parent compounds and metabolic products at specific intervals following applications. Rates of degradation are determined by bioassay and chemical analyses in eggs and tissues of the birds.

Studies are in progress on the effects of insecticides and management practices on the natural enemies of pest flies. Cultural practices which favor the biological control agents are integrated with selective applications of insecticides to minimize or prevent the destruction of natural enemies, but still obtain maximum kills of pest flies.

Research on external parasites of poultry also includes detailed studies to identify vectors of diseases. Suspect vectors are reared and fed on infected birds and transferred to healthy ones. Life history studies are performed on many of these insects.

There are 3.5 man-years committed by the States to research on poultry insects.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology, and Nutrition

1. House Flies. At Gainesville, Fla., various methods of tagging house flies have been evaluated and developed to study biology, dispersion, and behavior. Studies have shown that an individual male may attempt to copulate with a female at least 6 times in a day. Releases of tagged house flies on Grand Turk Island revealed the following house fly biology, **behavior** and dispersal: Flies dispersed in all directions from where they were released. In some cases flies remained around the release point for 1 or 2 days; in others they dispersed within a day. Using tagged flies it was shown that grid counts in buildings on Grand Turk represented approximately 1 to 10% of the population. In another study the ratio of untagged males to untagged females was 1.5:1. The total number of flies on the island was determined to be between 200,000 and 300,000. These represent present survivors of the chemosterilant bait applications which are being conducted on the island.

Studies have shown that emergence of house flies from the pupal stage exhibited circadian rhythm. A postulate has been made that emergence begins 12 hours after the onset of darkness. However, this response can be modified by the light regime of the parent generation, but is not obscured in toto. Preliminary data indicate that house fly susceptibility to DDT and diazinon also exhibited a circadian rhythm.

In Oregon, studies on the physiology and biology of house flies were continued. A physiological mutant strain of the house fly was isolated in which the majority of females emerge before any males appear. Strains such as this should prove useful in biological and chemosterilization studies.

Further characterizations have been made of enzymes responsible for organophosphate resistance in house flies. The mutant aliesterase responsible for organophosphate resistance is a simple esterase. Activity of the enzyme was not affected by any of a number of metal ions or by chelating agents. Using centrifugation techniques, 50-fold purification has been achieved. Breakdown of organophosphorus insecticides by the partially-purified enzyme is inhibited by organophosphate synergists such as DEF. Studies utilizing disc electrophoresis have shown distinct differences in proteins and esterases between several susceptible and insecticide resistant strains. Evidence for allelism of the genes controlling resistance to two organophosphorus insecticides has been obtained. Also research indicated that the major genes responsible for malathion, parathion, DDT, and Isolan resistance in house flies are all carried on the same chromosome and that genes for resistance to parathion and malathion are alleles.

In Oregon extensive studies were continued on the biology, nutrition, and colonization of the little house fly (Fannia canicularis). Vermiculite was preferable to wood shavings in the larval medium, increasing ease in handling, facilitating separation of pupae, and reducing mite infestations. A ball of 2-day-old alfalfa meal and water enclosed in a white muslin cloth is the basis for a new method of obtaining eggs. The moist ball is placed on filter paper in a petri dish; about one-fourth of the filter paper surface is available for oviposition to the flies which prefer it to the muslin. Eggs are easily removed and an average of 300,000 eggs are collected within a 4-hour period, twice weekly, by this method. In further studies, little house fly pupae survived refrigeration at 41°F for 20 days; emergence of adults from pupae held at this temperature for 32 days was only 70% as great and most of the flies were smaller in size. Adults emerged in good numbers from pupae held 24 hours at 23°F, but none emerged from pupae held at 23° for 4 days.

2. Mosquitoes. At Gainesville, Fla., studies on mosquitoes have been continued to elucidate basic biology fundamental to development of new and effective control techniques. Extensive laboratory and field research has shown that males of a laboratory colony of Anopheles quadrimaculatus do not disperse as readily as do males of the wild type. Laboratory crosses accomplished through forced copulation of the laboratory and wild strains have developed a hybrid strain which mated readily in the laboratory in the F₃ generation. Field studies on the wild strain of quadrimaculatus in Central Florida showed this species to consistently and predominately consist of young females (females in the process of laying their first batch of eggs). The only factor influencing the size of the population was the availability of larval breeding sites. Eggs of this species of mosquito could be stored at 58°F and 100% RH for seven days without decreasing their viability.

At Corvallis, Oreg., studies were continued on the biology and ecology of mosquitoes. The occurrence and distribution of Aedes increpitus in the Willamette Valley was followed by sampling areas in Suver community considered representative of larval breeding sites in the Willamette River from Eugene to Portland. All larval instars were found at weekly intervals from January through March 1964.

Laboratory studies in Oregon showed survival of Aedes sierrensis larvae for at least one year. Thirty-one individuals survived when maintained at temperatures of 41° to 46°F. When the temperature approached 46°F a few of the larvae pupated and adults emerged. At the end of the year all individuals pupated or emerged from pupae when removed to room temperatures.

Studies on mosquito biology were continued in California. Continuous rearing of Uranotaenia anhydor in the laboratory was accomplished. Eggs hatched in 2.5 to 3.5 days (75°F), larval and pupal stages required 2-3 days each with little mortality noted (77°F). The maximum life spans of a male and female

were 49 and 55 days respectively. Mating occurred readily and the preovipositional period was 4-5 days at 70°F.

Studies have led to a much better understanding of the ecology of Orthopodomyia californica. Contrary to suggestions in the literature, there appears to be little opportunity for competition between O. californica and Aedes sierrensis because of their different ecological preferences.

O. californica is not a rare species. Larval stages were collected every month of the year. Overwintering occurs at 2nd - 4th instar larvae.

O. californica was associated with constant or decreasing water levels in tree holes of cottonwood and willow which are phraetophytes. Aedes sierrensis require fluctuating water levels for egg hatch. In California, Orthopodomyia californica females fed on cotton pads saturated with blood of chicken, rabbit, or man, diluted with 5% glucose. The females laid eggs when blood fed, but not when deprived of blood, indicating that blood is necessary for oviposition. However, another mosquito, Uranotaenia anhydor could not be induced to feed on chicken.

Studies have confirmed that the major factor responsible for DDT-resistance in the mosquito, Culex tarsalis, is the increased ability to detoxify DDT by oxidative metabolism.

B. Insecticidal and Sanitation Control

1. House Flies. At Gainesville, Fla., selected toxicants were tested as contact sprays against house flies of the regular (susceptible) and/or Cradson (multi-resistant) colonies. Dimethoate was the most effective against both colonies. Diazinon and ronnel were superior to the malathion standard against both colonies, but diazinon was the better of the two against the regular colony and slightly less effective than ronnel against the Cradson colony. Hercules 9326 and ENT-27160 compared favorably with the standard malathion against the regular colony flies.

At Corvallis, Oreg., research has been continued on the development of synergists for overcoming house fly resistance to organophosphorus insecticides. Tests with ethyl, propyl and butyl DEF have shown that propyl DEF is an effective synergist for parathion against parathion-resistant house flies. A series of unsymmetrical esters of phosphoric acid contained materials highly effective as synergists for malathion against resistant house flies. In addition, various dialkyl analogs of parathion and malathion were effective synergists for the parent compounds. House flies selected for resistance with synergized malathion became resistant more slowly than flies selected with malathion only. Malathion-resistant house flies degraded Cl^4 malathion 2 to 14 times and excreted metabolites 2 to 4 times more rapidly than susceptible flies. Salithion was effective as an insecticide against susceptible and organophosphorus resistant house flies and was also effective as a synergist for parathion against resistant flies.

In Florida, 32 insecticides were tested as house fly larvicides in manure under caged poultry. One to 2 gallons of water emulsion of the insecticide were used per 1,000 ft² of manure, to give insecticide dosages from 100-200 mg/ft². Larval density was estimated by a new and highly accurate technique 1 day before and at intervals after treatment. Dicapthon caused complete mortality of the larvae within 7 days in the only test run with this insecticide (200 mg/ft²). At 200 mg/ft², Shell Compound 4072 killed all the larvae and at 100 mg/ft², gave 90% control after 7 days. In another test at 200 mg and in 2 other tests at 100 mg/ft², the insecticide reduced fly breeding by only 13-42%. Ethion at 100 mg/ft² gave 90% control after 7 days. Dimethoate, Bayer 39007, and Hooker HRS-1422 also produced considerable control of the fly larvae.

Of three colonies of the little house fly maintained in Oregon, the one most recently colonized showed indications of the greatest resistance to malathion and heptachlor which had been used from time to time at the poultry house that was the source of the three strains. Although ronnel had also been used at the poultry houses, no more than 2-fold resistance to this chemical could be found in the colonies.

2. Mosquitoes. Studies were continued at Gainesville, Fla., on the development of insecticides for the control of mosquitoes. These studies included screening of candidate chemicals against mosquito larvae and adults and laboratory and field evaluation of promising materials. In screening tests with Anopheles quadrimaculatus larvae, 70 of 226 compounds were considered effective enough to warrant further evaluation. In screening tests with Aedes taeniorhynchus adults 28 of 174 compounds were equal to or more effective than the standard insecticide, malathion.

In field tests of airplane spray applications of water emulsions or oil solutions for adult mosquito control, Bayer 41831 and Bayer 39007 reduced the adult population of Aedes taeniorhynchus and A. sollicitans by 99% at an application rate of 0.1 lb/acre and were more effective than malathion at the same application rate.

Testing of compounds to determine the systemic toxicity against mosquitoes has been continued. Thirty-five compounds previously tested for systemic action in rabbits against body lice, were evaluated in rabbits against Aedes aegypti mosquitoes. Three of these materials (Bayer 30468, Hercules 7845-C and Rhodia R.P. 9895) caused complete mortality to at least one lot of mosquitoes fed within 5 hours after treatment (25 to 100 mg/kg) without noticeably affecting the rabbits.

At Corvallis, Oreg., fenthion applied in granular formulations as a mosquito larvicide in log ponds was effective for 7 to 11 days. In laboratory tests, a series of unsymmetrical esters of phosphoric acid contained materials highly effective as synergists for malathion against resistant mosquitoes.

At Fresno, Calif., in field tests against Culex p. quinquefasciatus, dichlorvos (30%) resin cylinders were lethal to larvae and adults at distances of 2 to 5 feet.

3. Lice and Mites. In Oregon three new colonies of poultry parasites were established. One was the chicken body louse; the other two were the chicken mite and Norco (California) strain of the northern fowl mite. (A previously colonized strain of the northern fowl mite is the Hansen strain).

In screening tests in Texas 7 commercial compounds were highly effective, giving 100% control of lice on poultry for 28 to 35 days. The most effective, Monsanto CP-40294, Shell SD-8436 and SD-8448, and Stauffer R-5725, were effective in sprays at a concentration of 0.05%, as was the malathion standard. In field tests, a flock of about 800 white leghorn laying hens was pen-sprayed. Before treatment, 35 or 40 birds examined had moderate to heavy infestations of the chicken body louse and some birds also had light infestations of the wing louse. The hens were sprayed twice, about a week apart, using 10 gallons of 0.25% Ciodrin for each treatment. At the time of the second spraying, only 3 of 40 birds examined had light infestations (less than 5/bird); 1 week after the second spraying, no lice could be found. On each of 4 subsequent weekly inspections, the birds were devoid of lice. In the treatments an attempt was made to spray under and upper sides of the birds, but it was obvious that not all the birds were thoroughly wetted by the spray.

In Oregon, the Hansen strain of the northern fowl mite showed an LD-50 of 0.0072% for malathion; with 3 candidate malathion synergists, the LD-50's were one-fourth to one-thirteenth as large, demonstrating high efficiency on the part of the synergists. The Norco strain of the northern fowl mite has a long history of exposure to malathion; after 4 years of usage malathion failed to provide control at levels of malathion above the recommended rates of application. Tests showed the Norco strain to be 3 times more resistant to malathion than the Hansen strain, with an LD-50 of 0.021% for the Norco strain. This is equivalent to $5.2 \mu\text{g}/\text{in}^2$ for the Hansen strain and $15.2 \mu\text{g}/\text{in}^2$ for the Norco strain. A malathion synergist, S,S,S-triethyl tritriophosphate restored the effectiveness of malathion, but boosted the efficiency even more for the Hansen strain. Northern fowl mites from the Oregon State University turkey farm also showed malathion resistance (LD-50 was 0.01%). The LD-50 for malathion synergized with ENT-25812 showed a 50-fold increase in toxicity over malathion alone. The newly colonized strain of chicken mites from the Oregon State University poultry farm was assayed for susceptibility to malathion and an LD-50 of 0.004% was indicated in this preliminary test ($7.2 \mu\text{g}/\text{in}^2$).

The Extension Service pointed out the need for control of chiggers on rangelands at the time turkeys are put on range. Currently recommended chigger control insecticides include chlordane, lindane, or toxaphene, but these cannot be used on range areas utilized by poultry or livestock, as they would cause residues in the meat. Malathion at 0.5 to 1 lb/acre gives

excellent initial control of chiggers, but effectiveness lasts only about 2 weeks. If turkeys can be protected for chiggers for the first 2 weeks, they will not suffer the downgrading of carcasses due to lesions from chigger attacks. Malathion is currently recommended for use in poultry houses and direct on birds for lice and other mites and it is now registered for application to turkey ranges just prior to release of the birds.

C. Insecticide Residue Determinations

1. Toxicity Studies. Research was conducted in Texas in cooperation with the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other chemicals.

Atropine, the standard therapeutic antidote against poisoning by organophosphorus compounds, acts by opposing the stimulation resulting from accumulation of acetylcholine but does nothing to treat the basic biochemical lesion or the inhibition of the essential enzyme, cholinesterase. A need for an antidote that would reactivate inhibited cholinesterase has been recognized for many years. Various oximes have been proposed and have shown beneficial action together with specificity toward both compounds and species of animal. In previous studies, the oxime dosages employed did not seem useful against coumaphos poisoning.

Although carbamate insecticides inhibit cholinesterase, as do organic phosphorus compounds, the process is by carbamylation instead of phosphorylation. Laboratory animal studies indicated that oximes such as 2-PAM intensified the action of carbaryl instead of reversing the enzyme inhibition. Phenothiazine derivatives have some potentiating effects in organic phosphorus insecticide poisoning.

Studies were continued to determine the hazards of chemosterilants to poultry. Previous reports have emphasized the radiomimetic effect produced by apholate, tepa, and metepa; particularly the deleterious effect upon the tissues that form white blood cells. Further studies have shown a second effect, teratogenesis - that is, the production of monstrosities and defects in the young of animals and birds. Incubating chicken eggs injected with the chemosterilants--apholate, tepa, or metepa at various times showed a disconcerting number of defective chicks. Defects included shortened upper or lower beaks, crossed-beaks, absence of legs, curled and fused toes, herniation of the brain, lack of eyes, schistosomus, and growth retardation. At high dosages the embryos died or did not begin development.

D. Biological Control

1. House Flies. A pathogenic fungus of the order Entomophthorales was found in the little house fly colonies in Oregon. About 85% of the adult flies were killed before the infected colonies could be isolated. Development of the fungus apparently requires about a week in the fly. Of interest is the fact that the fungus tolerates a wide range of relative humidities, from

30% to 70%. Attempts to infest house flies with this species of fungus were unsuccessful. After about 3 months colonization the fungus strain was lost in spite of attempts to maintain it. Attempts to culture the fungus on an artificial medium (potato-agar) were unsuccessful. The method that was successful for 3 months relied upon transferring infected flies to cages of clean Fannia canicularis.

2. Mosquitoes. Cooperative studies in California have been conducted on biological control agents for mosquito larvae. Many larvae of Aedes ventrovittis and A. hexodontus and a few larvae of Aedes cataphylla and Culex tarsalis infected with microsporida were collected in June 1963 near Tenaya Lake in Yosemite National Park. Although infected larvae of some species were relatively abundant, the infected portion of the population was estimated at less than 1%.

An epizootic of possibly a microsporidian (not of the genus Thelohania) was observed in several large swales in which Aedes ventrovittis larvae were present in large numbers. Many dead larvae were present and most larvae were visibly affected. Some larvae were pupating but in the laboratory most pupae died or the adults failed to emerge. Identification of the pathogen and its relationship with the host is being undertaken.

A bacterium (Bacillus sphaericus) prepared by the Bioferm Corporation has been evaluated with success against many mosquito species in laboratory tests. Limited field trials against mosquito larvae breeding (Culiseta incidens, C. peus and Aedes sierrensis) in rock and tree holes were conducted by treating these with the bacteria. Water temperature ranged from 38° - 48°F. No effect of the bacteria on the larval population was noted. It is possible that the cool temperature prevented build-up of the bacteria.

E. Insect Sterility, Attractants, and Other New Approaches to Control

1. House Flies. Tests were conducted at 3 poultry farms in Hernando County, Fla. to evaluate the effectiveness of 2 chemosterilants, metepa and apholate, for control of house flies in comparison with an insecticide, trichlorfon. Semiweekly bait treatments with 1% of the chemicals in sugar-water solution were applied to manure beneath the cages. Male and female flies were collected in the poultry house for sterility determination. The poultry house treated with metepa bait showed little fly reduction for the first 2 weeks after treatment, but the flies progressively decreased for the next 3 weeks. In the following 2 weeks, there was a slight rise in fly counts, after which there was such a drastic drop in fly production that within 1 week no flies or breeding could be observed, and the treatments were discontinued. During the next 2 1/2 months, only 3 flies were seen and they may have flown into the area or have been carried there. Male sterility was usually above 90% and the female sterility was often 100% until the 7th week, after which the sterility tests had to be discontinued for lack of flies. The fly population at the poultry house treated with the other chemosterilant, apholate, remained fairly low and constant for the first

6 weeks, then dropped for 2 weeks. However, the population increased slightly and remained at the early posttreatment levels for the rest of the test. Sterility induced in both sexes was rapid and nearly complete, and the lack of control was believed due to infiltration. The flies were not eradicated as they had been in the poultry house treated with metepa. Fly densities at the poultry house treated with insecticidal bait (trichlorfon) decreased immediately after treatment and remained at about the same low level throughout the test period.

In Oregon studies were continued with the little house fly. With adult flies, residues of tepa were more effective than metepa, which was more effective than apholate. Tepa was also more effective when fed to the flies. The minimum amount required in the diet was about 0.01%. In a residual treatment, tepa was effective as low as 0.1 mg/ft². Pupae dipped for 1 minute in metepa produced flies with no detectable sterility, even at a concentration of 5% metepa. In cage tests, blue strings (wool yarn) impregnated with 1% solutions of metepa caused considerable sterility of this fly, but higher dosages would be needed for full sterility. Very few eggs were deposited when the strings were treated with 5% metepa and there was no hatch. No eggs were deposited in cages with strings treated with 10% metepa solutions. The metepa-treated strings were not repellent to the flies since as many landed on treated strings as on control strings.

In other tests male little house flies were more susceptible to sterilization by metepa than females. Preliminary mating competitiveness studies with the little house fly exposed to residual treatments of metepa at 10 mg/ft² look encouraging.

Tests to discover attractants for the little house fly continued in Oregon. None of 45 compounds screened as attractants in the field caught more than 5 flies. By contrast, fish fillet flour caught 840, defatted liver caught 484, and fish flour caught 464 flies. A large percentage of the flies captured were females. Fish flour was the most attractive of five materials tested in the laboratory, capturing 96% of the flies caught. In further tests, whole fish flour, defatted liver, and the flour made from fish fillets were oviposition stimulants. In other tests, little house fly adults alighted on blue and red strings in preference to green, yellow, and white strings.

2. Mosquitoes. At Gainesville, Fla., 22 compounds were tested as sterilants against larvae of Aedes aegypti. Only two of these compounds caused sterility. In other tests hempa at 50 to 100 ppm gave 99% sterility and ENT-50664 caused complete sterility in a few adults that survived the 5 ppm treatment. Feeding adult aegypti mosquitoes with seven candidate chemosterilants showed three of these to be highly effective.

Aedes aegypti larvae and adults were treated with apholate and tepa to determine if the males would recover fertility after successive matings. Recovery of fertility was almost complete by the 4th mating with males

treated as larvae with apholate. Males treated as larvae with tepa showed less recovery of fertility. There was no indication of recovery of fertility in males treated as adults with residues of tepa.

Studies were conducted with Aedes aegypti mosquitoes to determine if resistance to the sterilizing action of apholate could be developed through selection of sub-sterilizing dosages. Selections were made by exposing larvae in treated water. Two colonies of aegypti selected over 5 to 10 generations developed resistance to the sterilizing action of apholate. Whether resistance would develop by treating adults of other species of insects cannot be predicted, but results indicate the possibility of the development of resistance which must be considered in the development of chemosterilants.

In Oregon ethylenimine, a breakdown product of tepa, at 10 ppm caused no mortality of larvae, but high mortality of emerging adults of Culex p. quinquefasciatus. A dosage of 14 ppm of tepa to a ground pool containing Culex peus prevented most adults from emerging.

In Oregon extracts of both sexes of Culex tarsalis and C. quinquefasciatus showed little if any attraction to mosquitoes of the opposite sex, though in one test an ether extract of female C. quinquefasciatus provided sufficient attraction to males of that species to warrant further testing.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Basic Biology, Physiology, and Nutrition

Rogoff, W. M., Beltz, A. D., Johnsen, J. O. and Plapp, F. W. 1964. A sex pheromone in the house fly, Musca domestica L. J. Ins. Physiol. 10: 239-246.

Insecticidal and Sanitation Control

Chapman, H. C. and Lewallen, L. L. 1963. Field test with insecticides against mountain Aedes larvae in Calif. Mosq. News. 23: 41-44.

Gahan, James B. 1962. Recent research on mosquitoes conducted by the Entomology Research Division, USDA, Orlando, Florida. Proc. 8th. Ann. Conf. Ill. Mosq. Cont. Assoc.: 1-9.

McDuffie, W. C. 1963. Research on mosquito control by the USDA--past, present and future. Proc. N.J. Mosq. Ext. Assoc.: 112-120.

Insect Vectors of Diseases

Anthony, D. W. 1963. Arthropod vectors of some livestock and poultry diseases. Proc. U.S. Livestock San. Assoc.: 588-596.

Insect Sterility, Attractants and Other New Approaches to Control

- Dame, David A. and Schmidt, Claude H. 1964. Uptake of metopa and its effect on two species of mosquitoes (Anopheles quadrimaculatus, Aedes aegypti) and house flies (Musca domestica). J. Econ. Entomol. 57: 77-81.
- Gouck, H. K., Meifert, D. W. and Gahan, J. B. 1963. A field experiment with apholate as a chemosterilant for the control of house flies. J. Econ. Entomol. 56: 445-446.
- Gouck, H. K. 1964. Chemosterilization of house flies by treatment in pupal stage. J. Econ. Entomol. 57: 239-241.
- LaBrecque, G. C. and Gouck, H. K. 1963. Compounds affecting fertility in adult house flies. J. Econ. Entomol. 56: 476.
- Meifert, D. W., Fye, R. L. and LaBrecque, G. C. 1963. Effect on house flies of exposure to residual applications of chemosterilants. Fla. Entomol. 46: 161-163.
- Murvosh, Chad M., LaBrecque, G. C. and Smith, Carroll N. 1964. The effect of three chemosterilants on house fly longevity and sterility. J. Econ. Entomol. 57: 89-93.
- Smith, Carroll N., LaBrecque, G. C. and Borkovec, A. B. 1964. Insect chemosterilants. Ann. Rev. of Entomol. 9:269-284.
- Weidhaas, Donald E. 1963. Highlights of research on chemosterilization of mosquitoes. Proc. 31st. Ann. Conf. of Calif. Mosq. Cont. Assoc.: 21-23.

EQUIPMENT AND BUILDINGS USED IN POULTRY PRODUCING
Agricultural Engineering Research Division, ARS

Problem. Modern methods of producing poultry and eggs call for increasing use of engineering principles. More knowledge is needed about the effects of environment on birds and their products and what structures and equipment are best suited to provide the most favorable environment. Better methods of applying pesticides are needed and methods of eliminating pests without using chemicals would be preferred. New and additional methods are needed for using electrical and other energy to replace costly human labor in many operations devoted to feeding and care of birds and their products. Manure disposal is becoming an increasing problem.

USDA AND COOPERATIVE PROGRAM

This is a continuing program involving engineers and architects conducting basic laboratory investigations, application of laboratory results to a production basis, and development of typical plans for poultry structures. The work is in cooperation with the Animal Husbandry, Animal Disease and Parasite, and Entomology Research Divisions of ARS, USDA, and is a contributing project to NE-8, "Essentials of Poultry Housing for the Northeast." Plan development work is cooperative with all the State Colleges through Regional Committees, and with the Federal Extension Service as part of the Cooperative Farm Building Plan Exchange. The professional man-years shown in parentheses at the end of each of the following sections may include work applicable to other species.

A. Poultry Engineering. Poultry house environmental design criteria are investigated in controlled-temperature laboratory studies at Beltsville, Maryland, in cooperation with AH, ARS, and the basic laboratory data are applied to experimental poultry houses of the NE-8 Regional Project for evaluation. (4.7 PMY)

B. Construction Standards, such as serviceability and safety, for design of farm buildings are studied at Beltsville, Md., and selected field locations. Liaison is maintained with the American Society of Agricultural Engineers, American Standards Association, National Safety Council, National Fire Prevention Association, and other organizations concerned with standards and safety in farm structures. (0.4 PMY)

C. Water Supply and Wastes Disposal for the farmstead are studied at College Park, Md., in cooperation with the Maryland Agricultural Experiment Station. Liaison is maintained with the Public Health Service, the Water Systems Council, the American Society of Agricultural Engineers, and other organizations concerned with rural sanitation. (0.4 PMY)

D. Radio Frequency Treatment of Salmonella in eggs is conducted at Pullman, Washington. (0.1 PMY)

E. Equipment and Control for automatic feeding of livestock and poultry is under development in Washington and Illinois State Experiment Stations. (1.0 PMY)

F. Poultry Environment Equipment. Research on equipment for basic and applied studies involving light and thermal environment for poultry is underway at Beltsville in cooperation with the Poultry Branch, Animal Husbandry Research Division. The influence of electric equipment and environment on health and disease is being studied in USDA laboratories at Athens, Georgia. (1.5 PMY)

PROGRAM OF STATE EXPERIMENT STATIONS

There is an extensive program of both basic and applied research underway at the State Agricultural Experiment Stations in an effort to provide the answers to the continuing series of questions being raised by livestock producers. Demands are being made for more information on the effects of environment on the physical well being of all classes of livestock, and the way such optimum environments can be economically achieved; on new approaches to meet the growing labor shortage; on methods to adapt existing structures and equipment for greater economy of production; and on structures and related equipment for improved efficiency of feeding and materials handling operations.

Studies are being made of the effect of environment on the health, growth, production and fertility of dairy cattle, beef cattle, poultry and swine. Equipment and systems for efficiently transporting feedstuff into and out of storages and automatically mixing and feeding complete rations are being developed.

Exploring methods for improved care and housing of farm animals with greater economy and labor efficiency are also in progress as well as investigation of ways to modify existing structures and equipment to meet present-day economic conditions.

A widespread research effort is in progress which is attempting to investigate all of the factors involved in the complicated problems concerned with disposal of farm waste materials, particularly concentrated manures resulting from confinement-type livestock operations. The problem is most acute and the public is demanding a fast solution to this unsanitary and potentially dangerous health hazard.

Studies are conducted to obtain information on uses of electrical energy and explore new uses and test equipment. Many of the projects are concerned with the varied problems of chore labor mechanization and an expansion of the use of electricity for ventilating, heating, lighting, and cooling under the various production enterprises of today's farming operations. Development and testing of prototype specialized equipment for

product collection, processing, packaging, and transport, as well as storage, loading and unloading devices, are a part of the overall program of investigations.

Approximately 45 professional man-years covering work on all animal species and poultry are devoted to these problems. Much of the research is conducted cooperatively with the Department.

PROGRESS - USDA AND COOPERATIVE PROGRAMS

A. Poultry engineering

1. Calorimeter studies. Investigations to determine heat and moisture production of broilers were continued in the respiration calorimeters at Beltsville, Maryland. Analysis of data from a series of 9-week tests of Athens Randombred broilers in the calorimeters at 5°, 10°, 25°, and 30° C. showed that the total heat production peaked at about 20 to 23 Btu/hr/lb. live weight between 12 and 15 days of age. In contrast, previous tests with growing NH chicks on litter showed that the heat production peaked 10 to 15 days later at about the same value. Development of the homeothermic ("body thermostat") mechanism in modern strains of broilers appears to be accelerated, and current brooding temperature reduction regime may need revision. Latent heat production ranged from 3 to 5 Btu/hr/lb. live weight near day-old age and gradually decreased to 2 to 3 Btu/hr. near 40 to 45 days of age. Sensible heat production ranged from 6 to 18 Btu/hr/lb. live weight near day-old age. The higher value was obtained at 95° F. and the lower one near 91° F. Huddling of chicks at the lower temperature may have decreased the heat output.

It appears that fecal moisture content of broilers is around 80 to 83 percent on a wet basis. Although the growth of the Athens Randombred broiler stock in the calorimeter was not spectacular, basic engineering data on water to feed ratio and water plus feed to fecal production will be usable. These data need to be applied to an instrumented broiler house to test the suitability of the laboratory data for engineering design data. In a 3-day test of broilers after the 9-week age, birds which had been at 51° F. and 75 percent RH panted when placed in the calorimeter at 77° F. and 76 percent RH. Another group of broilers, which had been at 77° F. and 76 percent RH, when exposed to 51° F. and 75 percent RH increased sensible heat output by about 1/3, and seemed to be "uncomfortable". Further analysis is needed to assess the implication of sudden weather changes in a broiler house.

2. Southeast poultry disease laboratory. The facility at Athens, Georgia, was just made available during the year so considerable engineering time was spent on design and installation of laboratory and shop equipment and instrumentation. A prototype cabinet, to be used as a laboratory tool in the study of the relationship between environment and

disease, was substantially completed and some performance tests were conducted. In addition, specifications have been prepared for materials and equipment required for operation of the laboratory buildings, but which were not included in the construction contract. These total in excess of \$100,000.

3. Field observation on relation of housing to disease in the South Central States. Work at State College, Mississippi, in cooperation with Animal Husbandry Research Division and the Mississippi Station, has continued.

Insulation in the roofs of broiler houses has had mixed effects on production results of broilers. Insulated houses did not always produce the largest average weight, the highest feed efficiency, or the lowest condemnations--largely because of differences in management techniques from one house to another. These differences make it impractical to draw firm conclusions from the data to date.

Orientation of the houses showed little effect on the production of broilers. The north-south house receives more direct radiant heat than the east-west house in both winter and summer. The average weight, feed conversion, and condemnations, however, have been about the same in both the north-south and east-west oriented houses.

Temperature data show that the brooder does not maintain a uniform temperature under the hover. This was true of the gas hover and the hot water brooding systems, which were the only ones studied. This could be caused by a faulty thermostat or one that has been in use too long and is not as sensitive as it should be.

Investigation of the effects of curtain materials on environment within a broiler house showed a wide variation in the amount of radiant heat that can be transmitted through the different materials.

4. Influence of turkey housing environment on disease. Work at St. Paul, Minnesota, in cooperation with the Minnesota Station, has continued. The original objective was to determine how varying environments affected the course of airsacculitis disease in turkeys. During the past year breakthroughs in egg dipping procedures have made possible the purchase of poultts free of the S₆ strain of Mycoplasma gallisepticum. The engineering objective of the project, therefore, has changed to one of maintaining a disease-free environment. This calls for isolation measures to prevent entry of disease from outside sources and elimination of environments that might produce stresses. Equipment studies are being conducted parallel to the disease studies.

Two flocks of broiler turkeys have been grown to maturity from dipped eggs. The last flock grown during the coldest season of the year had few condemnations, none from airsacculitis. A salmonella outbreak was finally traced to field mice which had moved into the building at the onset of cold weather.

High concentrations of ammonia remain a problem. The addition of supplemental heat has been necessary in all pens to keep the litter in acceptable condition. The higher temperatures, however, seem to favor the production of ammonia. The drying of the litter, although better at an elevated temperature, never got the litter dry enough to decrease the ammonia production.

Several types of brooders have been tried in the course of the experiments. Losses have varied from 1 percent to 40 percent. Homemade hovers over hot water finned radiators and infrared heat lamps have given the best performances so far.

5. Plan development. No plans for poultry structures were prepared during the year.

B. Farmstead manure disposal. Laboratory and field studies are continuing in Maryland, in cooperation with the Maryland Agricultural Experiment Station, on the characteristics of animal manures that affect their handling and disposal and on developing design criteria for disposal lagoons. Laboratory work has shown that a potable, sanitary "water" can be produced from manure lagoon effluent by chemical disinfection. The process should be within the means of many farmers. Observation of soil sealing and sludge buildup rates in an operating hog manure lagoon in Maryland substantiated previous laboratory findings of 39 days sealing time in a "Manor" soil and 1 mm. per month sludge buildup. Preliminary investigation of the effects of irradiation of lagoon liquids with radio-isotopes indicated that it is apparently possible to sterilize the liquids with low-level radiation and that algae cells are rendered non-reproductive for varying periods.

The major portion of a manuscript for a publication on farm animal manure disposal was prepared.

C. Turkey feeder location. At St. Paul, Minnesota, a cooperative study with the Minnesota Agricultural Experiment Station has been set up to investigate factors which may influence the arrangement, number, and location of the feeding equipment and subsequently the labor requirement in caring for turkey broilers. In a turkey facility having feeders arranged according to best available recommendations, initial observations of feed use per feeder give indication that about 30 percent of the feeders are used very little. Also that the pattern of feeding concentration is not

consistent but may change for no apparent reason. Exploratory investigations tend to show the possibility of some correlation between litter moisture content and feeder non-use in specific areas of the pen. This factor as well as others including ammonia production, air temperature and waterer location will be studied to determine their influence on the arrangement and number of feeders necessary. If the influencing factor can be determined and corrected a reduction in the recommendations for feeder numbers and location may decrease the amount of labor required for feeding as well as for servicing the feeding equipment.

. D. Glow-discharge Treatment of Dried Egg for Salmonella

Experiments were conducted at Pullman, Washington to determine the effectiveness of glow-discharge radiation in reducing the numbers of Salmonella in dried egg powder. Samples of diatomaceous earth (celite), dried egg albumen, and dried whole egg were inoculated with the bacteria and then irradiated. The results indicated complete destruction of Salmonella in the celite and a marked reduction in numbers in the albumen, but little, if any, reduction in numbers in the whole egg powder. The results of physical and chemical tests conducted on the irradiated egg product indicated that no appreciable amount of damage was done to the product. This work will be continued in cooperation with the Animal Science Department of Washington State University.

E. Poultry Equipment

Studies involving a completely enclosed small automatic egg-sensing switch for automatically recording time of lay showed 327 perfect recorder marks of a possible 339 operations by a group of 20 switches. Three of the 12 errors were the result of switch malfunctions. The other nine resulted from eggs not tripping the switch, improper positioning of the switch or human error.

F. Equipment for Poultry Environmental Studies

At Beltsville, in cooperation with the Poultry Husbandry Branch, AHRD, this year's study completes the second year of a tentative 5-year program of orienting laying hens to an 18-hour diurnal environment where the temperature is maintained at 78°F. during the photoperiod and 62°F. during the dark period. Egg production during the 1962-63 study from the 60 highest layers was 3% (88% to 85%) lower than the highest 60 layers from the 24-hour control group. Results of the 1963-64 study show a difference of only 1.4% fewer eggs (86% to 84.6%).

During 1963-64, two studies of 144 Japanese Quail hens in each of the 18-hour and the 24-hour environments were completed. Egg production by Japanese Quail hens starts at 36-42 days of age, therefore, theoretically, three

studies of three 30-day laying cycles involving Japanese Quail can be completed during one cycle for chicken hens involving six 30-day laying periods. However, fertilization problems were encountered, thus reducing the season's work to one completed study. During this study the 18-hour quail hens laid 3.1% fewer eggs than their 24-hour controls. It is evident, also, from this study, that Japanese Quail are not stimulated to egg production by photoperiods shorter than 12 hours when placed in an 18-hour diurnally controlled environment of temperature and light.

Studies in cooperation with the Poultry Husbandry Research, AH Branch, of practical lighting systems for turkey breeding stocks have been completed. This year's work has been devoted entirely to preparation of publications describing and discussing the studies.

In Georgia, two trials were conducted in the altered air velocity facility to determine whether broilers 3 to 5 weeks of age would respond as favorably to increased air velocity as those 6 to 7 weeks of age had been found to do. Data obtained in these trials suggest that broilers as young as 3 weeks of age also grow faster and consume less water as the air speed in a hot humid environment is increased from 20 to 500 feet per minute. There were indications that air speeds higher than 500 feet per minute may reduce the growth rate and feed efficiency of broilers under 6 weeks of age.

Tests on the prototype environmental cabinet have indicated satisfactory performance of control systems for roof, wall, and floor temperatures and that further development is required to achieve satisfactory performance of control systems for air temperature and humidity.

PUBLICATIONS - USDA AND COOPERATIVE PROGRAMS

Poultry Engineering

Griffin, Jack G. 1963. Trends in broiler housing. Broiler Business, Volume 14, No. 4. April.

Griffin, Jack G. 1963. Liquefied petroleum gas as a brooder fuel. ARS 42-81. July.

Ota, Hajime and McNally, E. H. 1963. Heat dissipation studies of Arizona and Beltsville bred strains of SCWL. Poultry Science 42:1289-1290 (abstract).

Ota, Hajime and McNally, E. H. 1963. Poultry studies with respiration calorimeters. ASAE Transactions 6:129-131.

Wastes Disposal

Eby, Harry J. 1963. Poultry manure disposal. Proceedings of the National Symposium on Poultry Industry Waste Management, Lincoln, Nebraska. May

Equipment for Poultry Environmental Studies

Drury, L. N., Brown, R. H., and Driggers, J. C. 1963. Performance of chick brooder types in uninsulated houses. Ga. Agr. Exp. Sta. Bul. N.S. 101.

Marks, H. L. and Lucas, L. M. 1963. Time of oviposition under "short days". Poultry Science. Nov. 42(6):1466-1468.

Marsden, S. J. and Lucas, L. M. 1964. Effect of short-day or low-intensity light treatments on reproduction of fall-hatched turkeys. Poultry Science. Mar. 43(2):434-441.

II. NUTRITION AND CONSUMER USE RESEARCH

NUTRITION AND CONSUMER USE RESEARCH

Consumer and Food Economics Research Division, ARS
Human Nutrition Research Division, ARS

Problem. The assortment and characteristics of foods available to consumers are constantly changing with the adoption of new production, processing, and marketing practices. Constantly changing also, as nutrition science advances, is our understanding of the nutritional needs of man and the manner in which these needs can best be met by food. To help meet the Department's responsibility to advise consumers on the quantity and variety of foods that will assure maximum benefit and satisfaction, research must continue on the nutritional requirements of persons of all age groups, and on the nutrient and other values of foods and on how to conserve or enhance these values in household preparation and processing. Periodic surveys of the kinds and amounts of foods consumed by different population groups and individuals also are essential for evaluation of the nutritional adequacy of diets and to give the guidance needed for effective programs in nutrition education. Information from such surveys provides assistance needed in market analyses for different commodities and in the development and evaluation of agricultural policies relating to food production, distribution, and consumer use.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program of research concerned with (1) nutritive and other consumer values of raw and processed foods as measured by chemical or physical means and by biologic response; (2) effects of household practices upon the nutritive values and inherent qualities of foods, and the development of principles and improved procedures for household food preparation, care, and preservation; (3) surveys of kinds, amounts, and costs of foods consumed by different population groups and the nutritional appraisal of diets and food supplies; and (4) development of guidance materials for nutrition programs.

The research is carried out by two divisions of the Agricultural Research Service -- the Human Nutrition and the Consumer and Food Economics Research Divisions. Most of the work is done at Beltsville and Hyattsville, Maryland; some is done under cooperative or contract arrangements with State Experiment Stations, universities, medical schools, and industry. The total Federal scientific effort devoted to research in these areas total 63.3 man-years. It is estimated that approximately 3.8 man-years is concerned with studies related to poultry products.

Human metabolic studies and the related exploratory and confirmatory studies with experimental animals and microorganisms concerned with defining human requirements for nutrients and foods are not reported on a commodity basis, though some of the work is applicable to this report. This basic nutrition research represents a total Federal effort of 26.7 professional man-years and is described in detail in the report of the Human Nutrition Research Division. Certain aspects of this research related to egg products are considered briefly in this report.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

Nutrient Value of Food

Food composition and nutritive value are most frequently related to indigenous agricultural products. Specific and locally grown raw products are being extensively evaluated for essential nutrients, especially in Hawaii and Puerto Rico. Much work is related to changes induced by growing practices, processing and storage.

The form of fats and lipids in food stuffs and the changes involved in processing and holding are receiving special attention as the role of different types of fat in human nutrition unfolds. Protein content and structure continue as active research areas.

Certain raw products are being evaluated for their significant vitamin contribution to nutrition. The effect of production and processing practices on vitamin content continues as an area of interest. Additionally, research has been directed toward the study of vitamins in food stuffs as affected by inhibitory and stimulatory factors.

The total program in this area includes 36 projects in 23 States and is comprised of 23.4 professional man-years.

Properties Related to Quality and Consumer Use of Food

In the area of food preparation, products are related to quality by some measure. Special measures characterize certain classes of products; i.e., vitamin assays, enzymatic activity, water binding capacity, and changes in structural tissues. Combinations of these are involved in the quality evaluation work reported.

The major research in product development is on the production, processing and storage of beef, pork, lamb, poultry and eggs. Variables which affect the initial product, include feeding regimens, age and breed, are under study. Conditions of processing relate to freezing temperature, storage temperature and time, shelf life, and the effect of light.

Other research includes the quality of meat tenderness as influenced by chronological age, post-mortem aging and in relation to connective tissue.

Food preparation research focusing on products for home use include: Heat penetration of meats and baked products and the chemical changes involved; microwave preparation of meats, fruits and vegetables, including the chemical alterations involved; and flavor characterization in frozen and stored products by means of vapor component identification.

Many of these same factors are under study in institutional preparation where the quantities involved impose special conditions.

This portion of the program includes fifty-two projects in twenty-one States and is comprised of approximately 50.1 professional man-years. This is a partial report of the State Experiment Station programs in food science and includes work undertaken by home economics departments. For research on food and fiber utilization see reports of the Utilization Research and Development Divisions.

Food Consumption and Diet Appraisal

The State program in food consumption and dietary appraisal extends the work of the Department to other segments of the population or to geographic areas not separately identified in the nationwide studies. Currently twelve States are contributing to this program. One regional project is designed to yield information regarding food purchase and consumption patterns of families with preschool children. This group represents about one-fourth of the households in the North Central Region where the study is being made. Food habits will be evaluated in terms of the children's dietary needs. This research will provide information useful to both consumer and market interests.

The State program in this area totals 22.2 professional man-years.

PROGRESS--USDA AND COOPERATIVE PROGRAMS

A. Nutrient Value of Food

Tables of food composition. Research for the newly revised Agriculture Handbook No. 8 "Composition of Foods...raw, processed, prepared" has been supplemented by further research during the year and adapted to the needs of special projects.

Formulas and procedures that were used in calculating the nutritive values of 250 food items commonly prepared at home are being summarized in a publication for special users, particularly therapeutic dietitians and medical research workers. A table showing average adjustments for vitamin losses during cooking has been developed and will be included in the publication.

Selected data from revised Handbook No. 8 have been made available in decks of punched cards and magnetic tape for research workers. Available in these forms are the data from Table 1, the nutritive values for 100 grams edible portion of the foods; from Table 2, nutritive values for one pound of food as purchased; from Table 3, selected fatty acids in foods. Arrangements have been made for the sale of the cards and the tape by a private data processing firm in Washington.

Tables for the Department of Defense have been prepared on the composition of 630 food items procured by the Defense Supply Agency for feeding military personnel. Values for the composition of foods developed for Handbook No. 8 and many additional values provided by the Department of Defense were used to develop the data needed for the numerous special food products meeting military specifications.

B. Properties Related to Quality and Consumer Use of Poultry Products

1. Heating procedures and eating quality of turkey. Research on the rate of heat penetration during roasting of stuffed turkeys and the quality and yield characteristics of the cooked meat has continued to provide a basis for standardizing procedures for consumer use. The eating qualities were generally good when turkeys were roasted at 325° F. to an endpoint temperature of 195° F. in the breast or 185° F. in the thigh. Covering the turkey breast with aluminum foil helped equalize the cooking rates of the breast and thigh meat.

Heat penetration into the stuffing was adequate to assure destruction of food spoilage organisms in turkeys roasted to 195° F. in the breast or 185° F. in the thigh providing the turkeys were held for 20 minutes after removal from the oven to attain their maximum internal temperature.

Yield of cooked, edible meat excluding skin was about 45 percent of the weight of ready-to-cook, unstuffed turkey. A report of these findings has been prepared for publication.

Research on the microbial and quality characteristics of turkeys stuffed and roasted under different conditions is in progress under contract in Indiana. Data on rate of heat penetration in stuffed turkeys roasted at 200°, 325°, and 450° F., with records of weight losses and quality characteristics, have been obtained. Work is in progress on microbial survival during cooking and cooling in stuffing previously inoculated with Staphylococcus aureus and vegetative and spore forms of Clostridium perfringens.

Studies were completed on eating quality and yield of light meat and dark meat turkey rolls roasted at 250°, 325°, and 400° F. to endpoint temperatures ranging from 161° to 212° F. Manuscripts presenting data from this research are being prepared for publication.

2. Food distribution program. Revision of the publication "Quantity Recipes for Type A School Lunches" (PA 631), was completed in cooperation with the Agricultural Marketing Service and the Fish and Wildlife Service, U. S. Department of Interior. This recipe card file provides 324 quantity recipes or variations and other information needed in preparing Type A lunches in schools participating in the National School Lunch Program. Recommendations on preparing, storing, and handling a wide variety of poultry, meat, cereal, dairy, fruit, and vegetable products were updated to take into account recent research findings and technology. New recipes were laboratory tested and taste panel evaluated, and all formulas and serving yields were recalculated in line with the 1964 revision of PA-270, Food Buying Guide for Type A School Lunches.

C. Nutrient Functions

Dietary carbohydrates. Shortened lifespan and acceleration of degenerative changes in the tissues of the rat have been reported previously to result from feeding a diet containing 25 percent egg in contrast to the results when a diet containing no egg or one consisting of 100 percent egg was fed. Thus it was apparent that the adverse response to diet was due not to egg alone but to the interaction of some of the ingredients in the diet containing 25 percent egg. Research recently completed has shown that the response to this particular diet may be significantly influenced by the type of carbohydrate fed and by the heredity of the animals under investigation. Two strains of rats (BHE and Wistar) were fed the egg-containing diet identical in all respects except that the 39 percent carbohydrate was supplied by sucrose, dextrose, or starch.

By 150 days, the level of fat and cholesterol in the livers of BHE rats was high, with the greatest elevation when sucrose was fed. Dietary carbohydrate had little effect on liver fat or cholesterol in Wistar rats. By 350 days, kidneys of BHE rats were enlarged and showed signs of structural changes, this organ still appeared normal in Wistar rats. The level of cholesterol in the serum of 150 day-old rats was low regardless of strain or kind of dietary carbohydrate. The level of cholesterol was significantly elevated, however, in the serum of 350 day-old BHE rats, with the highest levels in the sera of rats fed sucrose. Serum cholesterol values were also elevated in 350 day-old Wistar rats but to a lesser extent than in BHE rats; no differences related to carbohydrates were seen.

BHE rats fed the sucrose diet had the shortest lifespan. Survival was significantly longer for all the other groups with no marked differences among them. Nephrosis, a type of kidney damage, was the cause of death of most BHE rats, and the acceleration of this condition by diet seems to relate most closely to fat deposition in the liver. The cause of death of Wistar rats varied and appeared to be uninfluenced by diet. These findings have been presented at scientific meetings and will be prepared for publication.

D. Food Consumption and Diet Appraisal

1. Planning for proposed nationwide survey, households and individuals.

A nationwide survey of household food consumption and of the food intake of individuals is scheduled for 1965. Plans have been developed for a survey that would provide at least 6,000 household schedules and 10,000 individual schedules in the spring of the year with smaller household samples in each of the three succeeding seasons. The information on the week's food use to be obtained from each household is similar to that obtained in 1955, except that information on home baking practices will not be requested and information requested on home food production, home canning and home freezing will be reduced to allow interview time for questions on the food intake of individuals in the households.

In preparation for the proposed first nationwide survey of the food intake of individuals, data obtained by recall on the 1-day intake of food from nearly 550 individuals of all ages in Washington, D. C. during June and July 1963, have been studied in relation to two controversial issues that concern collection of data. The survey findings indicate that for this group: (1) the nonresponse rate on food intakes from individuals is not influenced by taking a schedule on household food consumption first in comparison to taking none, nor is it influenced by taking a schedule on food intakes from half in comparison to all individuals in the family; and (2) homemakers report the amounts of food eaten by family members in terms of their individual servings far more often than as proportions of household amounts. Tabulations of the Washington data also are useful as a pretest for tabulation of the nationwide survey.

2. Effects of food distribution programs on diets of needy families.

A survey of the food consumption of more than 800 households that were not participating in the food stamp program in St. Louis was made in May and June 1964 to determine the relation between usual family food expenditures and payments required for food coupons. Homemakers were asked also why their families did not participate in the program. Results of the analysis will guide the Department in revamping the St. Louis stamp program to make it more acceptable to eligible families and yet keep it within the limits of the program. Because of interest in the nutritional quality of food consumed by low-income families, an assessment may be made later of the dietary levels of these families. This is the sixth in a series of USDA food program surveys made in cooperation with the Marketing Research Division, ERS to assist the AMS to administer the food stamp and direct distribution programs.

3. Food consumption of the rural population in Spain (PL 480 research).

A survey of the food consumption of the rural population in Spain has been initiated by the Spanish Ministry of Commerce under the cooperative sponsorship of the Economic Research Service and the Agricultural Research Service,

using PL 480 funds. The study will provide information needed in appraising potential markets in Spain for U. S. farm products and should yield information useful to U. S. authorities on efficient ways of improving nutrition in low-income areas. The Spanish Ministry of Commerce expects to obtain much useful information on which to base a program for improving the diets of rural families, especially through better distribution of food. Information on food consumption, income levels, and related socio-economic characteristics has been obtained from about 1,200 rural families in 6 major regions of Spain. In summarizing the results, emphasis is being placed on (1) determining the nutritional shortages among these rural families at different income levels in the different regions, and (2) computing income elasticities for different foods as well as total food consumption.

4. Nutritive value of national food supply. The nutritive content of the per capita food supply is calculated each year from estimates of quantities of foods consumed (retail weight basis) as developed by the Economic Research Service. This series, which begins with the year 1909, is being completely revised to incorporate newest estimates of per capita consumption, revised food composition data from Agriculture Handbook No. 8, and new information on the nutrients added to foods by enrichment and fortification.

A survey conducted by the Bureau of the Census for the Consumer and Food Economics Research Division has provided information for the years 1957-61, on quantities of enrichment ingredients supplied to processors to fortify flour and cereal products. Through this program about one-third more thiamine, one-fifth more iron and niacin and one-tenth more riboflavin are added to the Nation's diet than would be available if foods were not enriched.

For the first time, the enrichment survey was extended to include information on the quantities of ascorbic acid and vitamins A and D added to foods, thus furnishing a base line for future surveys. Currently the amount of ascorbic acid added to foods would be enough to increase the level in the per capita food supply by 5 percent. The contribution from synthetic vitamin A is 7 percent of which 6 percent is added through margarine. Vitamin D is not at present included in nutrient estimates.

5. Household practices in home freezer management. Recording forms and questionnaires for obtaining data on management practices of urban and rural home freezer owners were pretested and necessary revisions were made in preparation for data collection among households in Fort Wayne and a nearby rural area. Information will be obtained in two seasons on the kinds, amounts, sources, prices, and turnover rates of frozen foods stored in the home. Such data will provide information needed to develop guidance materials for improved management of home freezers.

6. Development of food budgets and other basic data for food and nutrition programs. Interpretation of nutrition research findings and their application to practical problems has continued as part of an ongoing program to assist nutritionists, teachers, health workers, and other leaders concerned

with applied nutrition programs or nutrition policies. Information developed under this program is provided to many groups both within and outside the Department working on practical food programs, on questions relating to nutritional requirements, food consumption, nutritional importance of specified foods, and on nutrition education. Increased emphasis has been given this year to opportunities for disseminating information to the public through TV and radio, the press, conferences, workshops, and the Department's Food and Home Fair.

Food budgets at different cost levels for individuals and families are priced quarterly for publication in Family Economics Review as a continuing service to welfare workers, extension agents, and others needing this information. For example, in June 1964, the cost of one week's food for a family of four including 2 school-aged children, was estimated to be \$24.40, \$32.80, and \$37.40, respectively, for the low-cost, moderate-cost, and liberal plans.

The food budgets published in Home Economics Research Report 20, "Family Food Plans and Food Costs," have been reexamined in the light of revisions in food composition data (Handbook 8, revised) and in recommended dietary allowances of the National Research Council. Some modification in food quantities was needed for certain individuals. This has necessitated revision of food plans and their presentation in technical and popular publications, including Agriculture Handbook 16, "Planning Food for Institutions," now being readied for publication. The "Food Purchasing Guide for Group Feeding," formerly a part of Agriculture Handbook 16, is in the final stages of editing for publication as a separate handbook.

All other existing guidance materials for nutrition programs were reviewed in light of the changes in recommended dietary allowances and in food composition data. Some publications have been revised; others will be updated for the next reprinting.

Nutrition Program News, a bimonthly periodical prepared for members of State nutrition committees and other community nutrition workers provides one channel for disseminating pertinent information about Federal programs and for reporting nutrition activities in the States. Issues this year included such diverse subjects as a report of the World Food Congress held in Washington, June 1963, "Labels on food products--the protection they give," and "Nutritional fitness for teenagers." Assistance to workers in nutrition programs has been provided also through consultation and program participation by staff nutritionists.

PUBLICATIONS--USDA AND COOPERATIVE RESEARCH

Nutrient Value of Food

Watt, B. K., and Merrill, A. L. April 1964. Composition of Foods...raw, processed, prepared. Agriculture Handbook No. 8. Revised. December 1963. 190 pp.

Watt, B. K. April 1964. Revising the Tables in "Agriculture Handbook No. 8." J. Am. Diet. Assn. 44(4): 261-263.

Leung, W. W., Pecot, R. K., and Watt, B. K. May 1964. Tables of the Composition of Foods for the Armed Forces. Department of Defense. Defense Supply Agency. 50 pp.

Consumer and Food Economics Research Division. Revised 1964. Nutritive Value of Foods. Home and Garden Bull. 72.

Consumer and Food Economics Research Division. 1963. Conserving the Nutritive Values in Foods. Home and Garden Bull. 90. 16 pp.

Properties in Relation to Quality and Consumer Use

Dawson, E. H., Brogdon, J. L., and McManus, S. 1963. Sensory testing of differences in taste. Part I. Methods. Food Technol. 17(9): pp. 45-48, 51, illus. Part II. Selection of panel members. Food Technol. 17(10): pp. 39-41, 43-44, illus.

Eisen, J. N. 1964. A note on orthogonal polynomials applied to treatment levels with unequal replications. Food Science 29(1): pp. 105-108.

1964. Food buying guide for type A school lunches. Agricultural Marketing Service; Agricultural Research Service; and Fish and Wildlife Service, U. S. Department of Interior cooperating. PA-270, 75 pp.

Food Consumption and Diet Appraisal

Adelson, S. F., 1963. A 2-week vs. a 1-week Reference Period in Surveying Diets of Individuals. Abstract in Proceedings of Sixth International Congress on Nutrition, Edinburgh: p. 477.

Consumer and Food Economics Research Division. 1963. "The Nutritional Review." National Food Situation, No. 106, Outlook Issue, Nov.

Friend, B. 1963. Enrichment and Fortification of Foods, 1957-61. National Food Situation, No. 106, Nov.

- Adelson, S. F., Delaney, I., Miller, C., and Noble, I. T. 1963. Discard of Edible Food in Households. Jour. Home Economics 55(8): 633-638.
- Redstrom, R. A., Davenport, E. and Murray, J. 1963. Consumer Practices in the handling and Storing of Commercially Frozen Foods, Two Cities, Two Seasons. Home Economics Research Report 23, 27 pp.
- Consumer and Food Economics Research Division. Cost of 1 Week's Food at Home. Family Economics Review. Sept., Dec. 1963; April, July 1964.
- Consumer and Food Economics Research Division. 1964. Family Food Budgeting...for Good Meals and Good Nutrition. Home and Garden Bull. 94.
- Nutrition Program News (periodical, 5 issues): July-Aug. 1963; Sept.-Oct. 1963; Nov.-Dec. 1963; Jan.-Feb. 1964; Mar.-June 1964.

POULTRY - PROCESSING AND PRODUCTS
Western Utilization Research and Development Division, ARS

Problem. The \$1.6 billion poultry meat industry operates on very narrow profit margins. This industry is confronted with the problem of converting continually increasing amounts of poultry into a wide variety of products having high quality and improved convenience, at costs attractive to consumers and remunerative to the poultry grower. More information on the properties and processing of poultry is needed to enable us to better utilize poultry in a variety of forms attractive to consumers. Increased utilization of poultry would also serve toward eliminating our feed grain surplus, increasing returns to farmers and providing better products for American consumers.

Although poultry is an efficient converter of feed to meat, more grain is used by poultry per calorie of food produced than by any other commercial animal because a high percentage of the poultry diet is grain and because poultry meat contains exceedingly little fat. Furthermore, one-fourth of all grain fed to animals is used for poultry and egg production. Hence, increased consumption of poultry products would be an effective means of increasing markets for surplus grain. Also, the efficiency of feed utilization by poultry makes possible low prices within reach of more consumers. A still further benefit would arise from the increased use of poultry by improving the nutrition of consumers having diets now low in animal protein.

The consumption of poultry has steadily increased from a 1947-1949 average of 22 lbs. per capita to 39 lbs. for 1963. This important increase has involved price, quality of product, availability, and disposable income. Because of the current low profit margin it is impractical to increase consumption by lowering farm prices. Increased demand for and consumption of poultry will require higher quality and more convenient products and a greater variety to meet the desires of the modern consumer. However, in addition to greater returns from increased demand, a greater profit margin for the farmer can, of course, come from greater efficiencies in processing.

The trend toward convenience foods and further processing has primarily led to precooked poultry products which are generally less stable, more subject to warmed-over flavors, and more likely to provide texture problems than uncooked items. With the expansion of operation and the emphasis on continuous, more efficient processing, need has arisen for improved processing procedures for feather removal, chilling, tenderization, freezing, deboning, and commercial cooking. Lowering the cost and improving the quality of products that can be stored at ambient temperatures, such as canned, dried, cured, and irradiated products, offer potential for poultry utilization in domestic and export markets. As a foundation for applied studies, further knowledge is needed on the chemical nature of flavor and flavor changes in

processing and storage, on tenderness development, and on proteins, lipids, and other components.

USDA AND COOPERATIVE PROGRAM

Basic and applied research on poultry meat and poultry meat products are conducted at the Division headquarters at Albany, California and by contract in East Lansing, Michigan, Madison, Wisconsin, and Berkeley, California. Fundamental studies on poultry flavor are concerned with the identification of flavor precursor constituents in poultry meat and in the isolation and identification of volatile flavor components developed during the cooking of poultry. The chemistry of muscle protein and post-mortem chemical changes are investigated relative to the tenderness and other quality characteristics of poultry. The basic physiological character of feather release mechanism in fowls is studied to provide a foundation for improved feather removal. Applied research is conducted on the stability of cold-tolerant organisms; special problems of flavor, texture and stability of precooked frozen foods; and processing factors that influence tenderness of poultry meat.

The Federal program of research in this area totals 14.9 professional man-years, including contract research equivalent to approximately 1.8 professional man-years per year. Of this number, 8.3 are assigned to chemical composition and physical properties; 6.6 to new and improved food products and processing technology.

PROGRAM OF STATE EXPERIMENT STATIONS

Both basic and applied researches on poultry and turkey meat and meat products are being conducted by State stations. A problem of major concern is preserving or improving the initial quality. This work is directed to maintaining quality during off-farm handling, killing, dressing, eviscerating, cutting-up, chilling and packing fresh poultry. The effect of the various operations on the product is measured and related to development of principles or design features which will best maintain quality. Factors such as wholesomeness, microflora, dressing percentages, condemnation losses, disease effects, physiological and biological changes during post-mortem aging, and tenderness are evaluated.

Efficient processing and utilization are dependent upon development of more efficient work methods, equipment and facilities for handling, processing and packaging poultry and poultry products. Pressures for high speed, continuous processing, i.e., continuous chilling, give added impetus to this research. Methods of feather release, removal and scalding are being studied. Radioisotopes are employed in investigations of blood removal. Equipment is designed, developed and tested. Effects on fat, on bone darkening, weight losses and nutritive value are followed.

A number of fundamental and applied studies are directed to elucidating the chemical and physical properties and composition of poultry and turkey meat.

Effects of diet, processing treatment, handling and storage on the various chemical components are measured and compared by following carcass lipids, protein and mineral content and distribution. Special attention is being given the biological value of the protein and other constituents. Other research deals with the specific ionic shifts associated with post-mortem changes in poultry meat.

Flavor and nutritional value are important attributes of poultry products. Investigations of the chemical composition of poultry products are being carried out to determine the chemical components of poultry products which are related to or produce flavor or off-flavors in poultry meat. Because of the adverse effects of off-flavors on consumer satisfaction, attempts are being made to reproduce conditions responsible for off-flavors and/or odors in market poultry in order to confirm the relationship to practices in processing or distribution and to devise means of control.

Basic microbiological studies are being directed to establishing the source of organisms, and the natural flora present on poultry; to the source and effects of organisms found in bruised or diseased tissue; to the build-up of organisms in the scald tank water and along the processing line; and to means for extending shelf-life by retarding bacterial growth in packaged poultry and in poultry products. Another phase of the work deals with growth, survival and control of potentially pathogenic organisms.

New products research is directed to accumulating fundamental information basic to development of new or improved poultry and turkey products. Studies include determining yield; methods for meat recovery; and methods for curing, smoking, cooking, and shelf-life extension of poultry products. New approaches are sought to improve form, texture, flavor, juiciness, and quality of products. Precooked, freeze-dried turkey and poultry products are being investigated.

Economic feasibility and market tests are being conducted to determine possibilities of commercial productions of new or improved products designed to increase the use of poultry. Economic efficiency of turkey and poultry processing operations are also evaluated.

A portion of the research on poultry products is conducted under the regional project NCM-7.

The total research effort in poultry and turkey products, processing and utilization is approximately 17.2 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Post-Mortem Biochemistry and Tenderness. Basic investigations are continuing on the proteins of poultry muscle and their reactions that affect the tenderness and other texture qualities of poultry meat. It was previously

shown that rate of muscle glycolysis correlated with and was possibly involved in tenderness achieved by aging slaughtered poultry. In the absence of glycogen, the meat was found to be more tender. Some causes of difference in glycogen level were found. The level was highest in birds that had been anesthetized before slaughter and significantly lower in birds immobilized by electric stunning during bleeding or allowed to struggle immediately before slaughter. The concentration of creatine phosphate, an important reactant in the glycolysis of post-mortem muscle, was found to parallel the glycogen level. Electrically stunned chickens had almost 90% less creatine phosphate than those that had been anesthetized before slaughter. Thus slaughter conditions may affect toughness.

The large variation in tenderness from one bird to another shortly after slaughter remains as a challenge to research. Rates of pH decline in chicken breast muscle immediately after slaughter also showed a considerable variation from one bird to another. However, when pH rate of decline and tenderness as measured by shear press were evaluated, the two showed no strong correlation. Processing efficiency could be materially increased if aging time could be reduced and this would be possible if handling and processing could be adjusted so that all birds had tenderness equivalent to the most tender ones. No empirical method for reducing aging time has been found and these basic investigations will continue to search for useful leads.

Correlations have been established between objective shear force measurements and subjective response of average panel members over the range of toughness in turkey meat that can result from inadequate aging. This now permits establishment of minimum aging times for a given required percentage of tender birds within a group, and also defines average acceptability in terms of an objective test.

2. Chemistry of Poultry Flavor. Basic research is continuing on the isolation and identification of volatile constituents of poultry and the relationship of individual compounds and groups of compounds to flavor. More than 200 compounds in the steam-volatile, isopentane soluble fraction of boiled chicken meat were indicated by fractionation with a sensitive gas chromatograph. By coupling the gas chromatograph to a mass spectrograph, about 30 compounds, comprising aldehydes, ketones, and hitherto unrecognized aliphatic and aromatic hydrocarbons, have been tentatively identified. Investigations also revealed that hydrogen sulfide evolves from the amino acids, cystine and cysteine, of muscle protein during cooking. Only about 1% of the amino acids was transformed per hour, hence the evolution of hydrogen sulfide will continue at nearly a constant rate for long periods of cooking. The rate of evolution was increased by increasing muscle pH. Hydrogen sulfide levels in chicken broth and meat were determined at various temperatures. Taste tests were conducted with samples at various natural hydrogen sulfide levels and samples to which hydrogen sulfide was added. Significant correlations between hydrogen sulfide level and chicken flavor have not yet been found but the investigation is continuing. Such an odorous substance might contribute to the flavor of cooked chicken even though much of it escapes during normal cooking.

3. Pharmacological Investigations. The poultry industry produces approximately 1 billion pounds of chicken backs and necks annually. Because of low market value of this material, it has been proposed that homogenized chicken backs and necks be incorporated in gravy, soup mixes, and new poultry products. Feeding tests with weanling rats were conducted to determine the availability of fluorine from ground bone meal prepared from dried chicken bones. Chicken bone meal was added to provide a range of dietary levels. For comparative purposes, identical levels of fluorine were provided in control weanling rat diets using sodium fluoride rather than the ground chicken bones. At the end of 190 days the results as measured by bleaching of rat incisor teeth were identical for the two sources of fluorine. These data support the contention that the fluoride in bone meal is as readily available as fluoride from sodium fluoride salt in the diet. The use of chicken back and neck bones for human food products must be evaluated relative to the total fluorine ingestion that is potential for any area or set of circumstances.

4. Physiology of Feather Release. The physiological mechanisms that control feather tightening and release are being investigated in contract research at Michigan State University in East Lansing. Further experimental evidence was found of muscle-controlled tension in feather follicles and its importance in establishing the force required to pull feathers. Anesthetization caused a 25-35% decrease in intrafollicular pressure which paralleled a decrease in feather pulling force. Mechanical or electrical stimulation of the nervous system increased intrafollicular pressures. Death of anesthetized birds by neck breaking led to a rise in intrafollicular pressure and feather pulling force.

Both sympathetic and parasympathetic nervous systems play a role in control of feather tightness. Sympatholytic and parasympatholytic drugs (agents which block or inhibit the respective nervous systems) produced decreases in intrafollicular pressure and in feather pulling force. Sympathomimetic and parasympathomimetic drugs (agents which imitate or stimulate the normal actions of the two respective nervous systems) were able to increase the intrafollicular pressure and feather pulling force of chickens in which the pressure and force had been reduced experimentally by anesthetization.

B. New and Improved Food Products and Processing Technology

1. Freeze-drying of Poultry. Two new vacuum dryer designs have been evaluated in preliminary studies. A fin tube dryer, in which heat exchange fins are located in the body of a rotating dryer so that the product tumbles against them during the drying operation, reduced drying time. New surfaces are continuously exposed to receive heat from the sources in the vacuum chamber. The dryer was installed, tested, and found to operate satisfactorily. An experimental model of a product-in-tube dryer was also built and proved satisfactory in preliminary trials. Use of input and outlet reservoirs gave a semi-continuous operation that was long enough to simulate continuous drying. In this dryer the product tumbles in tubes surrounded by the heat source (hot air, steam, or hot water).

These two new dryers plus the conventional vacuum shelf dryer and the tumbling dryer, constructed sometime ago, provide a wide range of heat transfer possibilities. Engineering data on freeze drying were obtained and will soon be available on a broad range not feasible before.

Commercial vacuum-dried poultry samples were evaluated. The products were inferior to fresh or frozen poultry meat in their dryness, toughness, and lack of cohesiveness. The defects were more noticeable in dark meat than in light meat. The procedures for organoleptic evaluation of these products were established and will be used in experimentally produced vacuum-dried poultry meat in search of ways to process chicken better.

A measurement of the expression of juice from poultry muscle tissue under standard conditions (Grau-Hamm press method) was applied to the measurement of juiciness and texture in raw, frozen thawed, cooked, and freeze-dried rehydrated poultry meat. Only a small sample (300 mg.) is necessary and the method lends itself to study of effects of processes on quality of freeze-dried poultry. The press characteristics of four commercial freeze-dried cooked poultry meat samples were quite similar.

2. Low-temperature Microbiology. Polyphosphates, used in the cured meat industry to improve texture and moistness, retard spoilage of raw poultry at refrigeration temperatures. Basic studies of the mechanism of the microbial inhibition showed that the polyphosphates inhibit by binding (chelating), and thus making unavailable, various trace elements required for growth. The inhibition was readily reversed by adding metal salts or by adding competing chelating agents that are small enough to enter the cell and be metabolized. Both the pyoverdines, which are chelators produced by certain psychrophiles, and peptone were effective. In small scale tests, treatment of raw chicken with polyphosphate solutions in a variety of ways increased the keeping time at 36° F. 20 to 70%. The cost of treatments thus far devised would be an important consideration where shelf life is the only benefit to be derived from the treatments.

An improved design was developed for our temperature gradient incubator that is used for the efficient determination of temperature limits of growth and survival of microorganisms.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

- Bailey, Glen F. and Nonaka, M. 1963. The infrared absorption spectra of the N-2 trans, 4 trans-dienaldehydes, C₅ through C₁₂. J. Applied Spectroscopy 17(6), pp. 156-158.
- Elliott, R. Paul and Straka, Robert P. 1964. Rate of microbial deterioration of chicken meat at 2° C. after freezing and thawing. Poultry Sci. 43(1), pp. 81-86.
- Elliott, R. Paul, Straka, Robert P., and Garibaldi, John A. 1964. Effect of polyphosphates on growth of pseudomonads from poultry meat. Bact. Proc., p. 1 (Abs. A4). (Abstract of paper presented at meeting of Amer. Soc. for Microbiology, Washington, D. C.)
- Milner, C. K. and Carpenter, K. J. 1963. The limiting amino acids of ground nut flour and meal as the sole protein source for the chick. Proc. of Nutrition Society 22(2): XXII. ^{1/}
- Ostmann, Orville W., Ringer, Robert K., and Tetzlaff, Marjorie. 1963. The anatomy of the feather follicle and its immediate surroundings. Poultry Sci. 42(4), pp. 958-969. (Contract)
- Ostmann, Orville W., Ringer, Robert K., and Tetzlaff, Marjorie. 1963. The effect of various neuromimetic, anesthetic and tranquilizing drugs on feather release. Poultry Sci. 42(4), pp. 969-973. (Contract)
- Pippen, E. L. and Nonaka, M. 1963. Gas chromatography of chicken and turkey volatiles: the effect of temperature, oxygen and type of tissue on composition of the volatile fraction. J. Food Sci. 28(3), pp. 334-341.

New and Improved Food Products and Processing Technology

- Elliott, R. Paul. 1963. Limitation of microbial levels in chilled and frozen foods. In "Microbiological Quality of Foods," Slanetz, L. W. et al., eds., Academic Press, New York, pp. 171-178.
- Elliott, R. Paul. 1964. Do frozen-thawed foods have a faster spoil rate? Frozen Food Fact Book, National Frozen Food Association, pp. 27-29.

^{1/} Research supported by P. L. 480 funds.

- Hanson, Helen L., Brushway, Marian J., Pool, M. F., and Lineweaver, Hans. 1963. Factors causing color and texture differences in radiation-sterilized chicken. Food Technol. 17(9), pp. 108-114.
- Klose, A. A. 1964. A look at basic research on feather removal. Poultry processing and marketing 70(2), pp. 13, 52, 54.
- Ostmann, O. W., Peterson, R. A., and Ringer, R. K. 1964. Effect of spinal cord transection and stimulation on feather release. Poultry Sci. 43(3), pp. 648-654. (Contract)

EGGS - PROCESSING AND PRODUCTS
Western Utilization Research and Development Division, ARS

Problem. The nearly \$2 billion egg industry is periodically faced with burdensome surpluses that drive prices below the break-even point for many producers. The industry is also faced with declining per capita consumption. The estimate for 1963 is down 18% from the 1947-1949 average consumption. Because the demand for table eggs is inelastic, the increased utilization of eggs must come in the form of new egg products that compete by means of quality and convenience. Adequate knowledge is lacking of the properties, processing characteristics, and new product potentials of eggs to develop new markets. Present outlets for the 10% of egg production that is frozen or dried include the baking, confectionery, salad dressing, noodle, and baby food trades. Modified and new products emphasizing quality and convenience are needed to increase acceptance of egg products by these industries in order to compete successfully with egg substitutes.

Increased utilization of eggs would not only benefit the producer, but would also diminish our feed grain surpluses since poultry and egg production account for about one-fourth of all grain fed to animals. Improved egg-containing products would benefit the producer in three ways: by providing an increasingly useful buffer for stabilizing egg prices; by providing additional uses and outlets for eggs; and by providing more remunerative outlets for wholesome eggs that are unsuitable for table use because of appearance or handling characteristics.

Egg processors have four general problems. First, the potential of yolk-containing solids in convenience foods can be fulfilled only with improvement of flavor stability, of dispersibility, and freedom from pathogenic Salmonella bacteria. Secondly, the processing costs of whites should be reduced and their utility improved. Third, further basic research on egg composition and components is essential to reach an understanding of physical and chemical changes induced by processing and storage and thus provide a rational basis for devising improved processes and products. Fourth, formulation studies designed to incorporate eggs into new household and institutional convenience products, are needed. This last study must encompass a full appraisal of physical, chemical, and microbiological problems peculiar to the formulated products.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, a broad program of basic and applied research is conducted at the Division headquarters at Albany, California; by contract in Ames, Iowa, Ithaca New York, and Davis, California; and by grant funds under P.L. 480 in France, Australia, and India. Fundamental research is conducted on egg proteins and their relations to the functional properties and quality of eggs, on egg lipids and their

role in off-flavor development in yolk solids, on the mechanism of bacterial penetration and survival in eggs, and on the bactericidal, antiseptic, anti-inflammatory, and food preservative properties of lysozymes and other components from eggs. Applied research is conducted on the stabilization of yolk-containing solids to increase the usefulness of eggs in dry mixes and other convenience foods, on new and improved drying procedures to make dried egg fractions and products more readily and more completely dispersible, on various methods of controlling Salmonella in eggs, and on factors in the handling of shell eggs that affect egg product quality and cost.

The Federal program of research in this area totals 16.1 professional man-years, including contract research equivalent to approximately 2.1 professional man-years per year. Of this number, 2.3 are assigned to chemical composition and physical properties, and 13.8 to new and improved food products and processing technology. In addition, three research grants on basic problems are supported by P.L. 480 funds.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations maintain a continuing program of basic and applied research on egg and egg product utilization. It begins with the concern for quality of the freshly laid egg and extends through to work designed to maintain the storage life of novel egg products.

Research on initial quality includes: evaluation of the influence of breed and strain of laying hen on the chemical properties of eggs and the relationship of these constituents to the functional properties of the egg; study of the effects of diet, such as the cause and prevention of egg yolk discoloration resulting from feeding cottonseed oil to laying hens; and determining the effect of various washing and shell treating techniques on the quality, nutritive value, stability and public health aspects of shell eggs and egg products. Other research is being conducted to determine the influence that shell characteristics, temperature, humidity, holding time in storage, and packaging have upon egg quality and use. The relationship of shell thickness and breaking strength to retention of initial quality and freedom from bacterial contamination is receiving special attention.

Basic investigations designed to more fully characterize egg composition and elucidate the relationships between chemical composition, structure, biological activity and functional properties are in progress. Studies are being made of the chalaziferous layer and of the vitelline membrane to determine roles each play in the migration of materials into and out of the yolk. A study of the comparative biochemistry of the proteins of eggs is going forward. Study of the protein fractions of eggs may lead to increased utilization of eggs by providing a better understanding of the factors affecting foaming, coagulative and emulsifying properties of eggs and egg products. Investigations of the cholesterol and other constituents and of factors affecting these constituents in eggs are providing basic data which are useful in considering health-related questions.

Microbiological investigations include: study of bacterial spoilage of shell eggs; determination of the mode of action of proteolytic and lipolytic enzymes of psychrophiles in the deterioration of eggs and the influence of shell membranes in resisting bacterial penetration; and investigation of means for control of Salmonella in egg products. Other work relates to methods for controlling bacterial contamination in new products.

Among the efforts to develop new egg products is work designed to develop shell-less eggs. Considerable attention is also being given frozen eggs and frozen egg products.

Designing, constructing and testing an experimental egg cleaning machine is a phase of the processing techniques research. Other processing research seeks to evaluate egg washing methods. Another study, which is searching for means to improve the quality of eggs from small plants, is engaged with developing and evaluating procedures for pasteurizing liquid eggs. Use of ultrasonic techniques for egg cleaning and processes for freezing eggs are also being studied.

Finally, the costs, efficiency and economic feasibility of egg processing operations are being studied. These engineering-economic studies consider alternative methods of utilization of eggs and the advantages of each.

A part of the work on egg utilization is conducted under the regional project NCM-7.

There are about 10.9 professional man-years devoted to egg utilization researches at State Agricultural Experiment Stations.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Oxidative Changes in Yolk Lipids. Contract research on the oxidative mechanisms involved in egg lipid systems was completed at the Hormel Institute in Austin, Minnesota. At present only organoleptic tests reliably detect oxidative flavor deterioration in yolk-containing egg powders. It was found that the increase during storage in amount of volatiles absorbing at 280 millimicron has potential as an objective index of flavor deterioration. Other objective measurements showed poorer correlation with the organoleptic results. The polyunsaturated fatty acids and neutral lipid fractions from stored eggs showed essentially no deterioration, while the intact phospholipid system showed measurable deterioration. Isolated egg phospholipid systems were found to autoxidize much more rapidly than methyl esters of highly unsaturated fatty acids. Hydrogenated egg lecithin promoted oxidation in a model system. These results emphasize the importance of the phospholipids in research on oxidative deterioration of eggs and other commodities also.

2. Egg Proteins. Basic investigations on lysozymes from various sources including eggs are continuing at the University of Paris, France, supported by P.L. 480 funds. Lysozymes are enzymes with bactericidal and other biological properties. Lysozymes were separated by ion exchange chromatography from turkey and goose egg white and other animal sources to compare them with lysozymes from hen eggs in amino acid composition and other characteristics. Relationships between chemical structure and biological activity of egg white lysozyme were investigated.

Research was initiated at the Indian Institute of Science in Bangalore supported by P.L. 480 funds to determine changes in properties caused by freezing of hen egg yolk proteins. A determination is being made of the specific changes in proteins and lipoproteins resulting from freezing and thawing hen egg yolk.

B. New and Improved Products and Processing Technology

1. Bacterial Spoilage of Eggs and Egg Products. Over 10% of eggs produced are utilized in frozen and dried products. Continuation of egg processing and its expansion in both domestic and export markets is essential to the economic soundness of the egg industry, but pathogenic Salmonella threatens the outlet and limits the expansion. Pasteurization to control Salmonella is under investigation. In these studies, Salmonella strains of average heat resistance at inoculations of 10^6 to 10^7 per ml. in whole egg were easily destroyed in conventional plate and holding tube equipment by treating at 140° F. for two minutes or by flash heating to 160° F. for approximately three seconds. Such conditions did not materially reduce the usefulness of eggs. Studies with a heat resistant strain, (S. senftenberg 775W), indicate that only higher temperatures or longer treatments would kill contamination that might occur. Three and one-half minutes at 150° F., which may be a practical limit of pasteurization for whole eggs, did not consistently and completely destroy a 10^4 per ml. inoculation of this strain. Steam injection heating for about three seconds at 165° F. did not harm the functional properties of eggs. Commercial-type pasteurization (142 to 143° F. for 3-1/2 min.) did not harm fresh whole egg even for the demanding requirements of sponge cakes. On the other hand, when frozen whole egg was pasteurized whip time had to be increased by a third to make an acceptable sponge cake. Its performance in other cakes, for example commercial-type sponge cakes, which are less dependent upon egg quality than true sponge cake, and layer cakes, was unaffected. Combinations of homogenization and careful control of heat pasteurization condition can be used to correct damage to the foaming power of pasteurized frozen whole egg liquid, but yield a much thinner product on thawing.

Pasteurization of unmodified liquid egg whites in commercial plate heater and holding tube equipment above 134° F. for two minutes caused an increase in turbidity and viscosity of whites. The most severe treatment that did not visibly change egg whites was 133° F. for two minutes, but this treatment damaged foaming power in angel cake. The loss in foaming power for angel

cake could be corrected by additives. The cake volume was unaffected. Treatment at 133° F. for two minutes is marginal and the absence of Salmonella from the final product would have to be verified bacteriologically.

Basic studies of the heat stability of individual egg white proteins were conducted in an attempt to find conditions that would permit egg white pasteurization by the whole egg procedure (140° to 144° F. for 3-1/2 to 4 min.). All of the proteins but one (conalbumin) were found to be adequately stable at pH 7 and this one could be stabilized sufficiently to withstand these conditions by addition of an iron salt or certain other metal salts. Aluminum salts were especially suitable because they are not toxic and they cause no color change as do the iron salts. Applied tests were conducted both in the pilot plant and in commercial plants. Excellent kills of salmonellae were achieved at 140° F. (as reported already for whole egg above) without coagulation or increase in viscosity of the white. Whip times were increased but could be corrected by adding certain conventional whipping aids. Angel cake volumes and textures were essentially unaltered.

Contract research on Salmonella contamination in eggs was initiated at Iowa State University at Ames. A technique for detection of Salmonella by fluorescent antibodies promises to give results in 24 hours comparable to the three- to four-day enrichment differentiation and confirmation tests now in use. Relationship of Salmonella infection in laying hens to egg contamination and relative contribution of egg infections and plant equipment sanitary levels to the presence of Salmonella in egg products are being studied.

2. Egg Powders. Addition of sucrose to whole egg and yolk before spray drying has long been known to yield powders with improved performance value and stability. However, at levels commonly employed, sucrose addition greatly accelerates and intensifies off-flavor development in air-packed powders even at refrigerator temperatures. Recently, in commercial practice, low dextrose equivalent corn syrup solids have been substituted for sucrose at the same level to improve flavor stability. This has led to the general belief that sucrose addition induces instability over a wide range of levels and, conversely, that low dextrose equivalent corn syrup solids do not. Present findings discount these beliefs and show that under mild oxidative conditions, comparable flavor stabilities and instabilities can be achieved with both sucrose and various corn syrup solids but at different levels of added carbohydrate. Typically, in each case, flavor stability was gradually improved at lower levels of added carbohydrates reaching a maximum flavor stability followed by an abrupt transition to marked flavor instability at succeeding levels. This transition corresponded to a change in the physical state of the egg lipids from one of coalescence where the lipids were readily extractable with mild solvent to a finely dispersed or emulsified state where the lipids were virtually nonextractable. Chemical indices of oxidative flavor deterioration (peroxide, carbonyl, TBA) correlated fairly well with organoleptic findings for powders stored at low temperatures.

Flavor and chemical stability relationships were determined for yolk, fortified whole egg, and whole egg solids as a function of graded levels of added sucrose, and 24 and 42 dextrose equivalent corn syrup solids. Use of corn syrup solids gave equally good protection as sucrose against browning reactions at elevated temperatures despite their containing substantial quantities of glucose and other reducing sugars. It is now feasible to select levels of any of the carbohydrates studied which yield dried products that combine good retention and stability of performance quality with outstanding flavor stability.

3. Precooked Frozen Foods. Studies of the effects of ingredients on stability of foam structure after freezing and frozen storage are continuing since successful freezing of such products would increase the utilization of eggs in this rapidly expanding industry. The effect of individual constituents on volume retention of products having a structure dependent on a stable egg foam is being evaluated. Foam stability was increased by increasing the solids content of whole egg souffles for freezing. Storage temperature was also important. At -30° F. products shrank, particularly those low in solids. Volume changed little at storage temperature of 10° F. but this temperature was unsuitable for souffles because they develop off-flavors within six months. Storage at 0° F. was, therefore, selected as optimum from both flavor and texture standpoint. Variations in formulas and baking conditions were studied for a dessert souffle made of egg white and sugar, to develop a product that was reasonably stable to freezing and storage. Addition of a sauce thickened with flour, similar to the type used successfully in a whole egg souffle, was not satisfactory in combination with an egg white foam.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

Garibaldi, J. A. and Bayne, H. G. April 28, 1964. Methods of washing eggs. U.S. Patent No. 3,131,067.

Jolles, Jacqueline, Jauregui-Adell, Juan, and Jolles, Pierre. 1963. Preliminary contribution to the study of the disulfide bonds in hen's egg-white lysozyme. Biochim. Biophys. Acta 71(2), pp. 488-490. ^{1/}

Privett, O. S. and Blank, M. L. 1962. The initial stages of autoxidation. J. Amer. Oil Chem. Soc. 39(11), pp. 465-469.

Windle, J. J., Wiersema, A. K., Clark, J. R., and Feeney, R. E. 1963. Investigation of the iron and copper complexes of conalbumin and transferrin by electron paramagnetic resonance. Biochemistry 2(6), pp. 1341-1345.

New and Improved Products and Processing Technology

Kline, L., Meehan, J. J. and Sugihara, T. F. Dec. 24, 1963. Process of spray drying eggs. U.S. Patent No. 3,115,413.

^{1/} Research supported by P.L. 480 funds.

III. MARKETING AND ECONOMIC RESEARCH

POULTRY PRODUCTS - MARKET QUALITY
Market Quality Research Division, ARSProblem.

Technological developments continue in the poultry industries and create many new problems relating to the market quality of poultry and egg products. Introduction of highly mechanized equipment and machinery plus new techniques in processing affect the absorption and retention of moisture of ready-to-cook poultry, the contamination of poultry and egg products by spoilage microorganisms, the physical damage to poultry carcasses, and the sanitary and functional properties of egg products. To maintain quality of these products in marketing channels, more information is needed regarding the effects of the new technology as well as changes that occur during transportation and storage. In addition, objective methods of quality evaluation are needed for use in developing improved criteria and standards for inspection and grading to insure uniform, standardized, and wholesome products.

USDA PROGRAM

The Department has a continuing program involving food technologists and bacteriologists engaged in basic and applied research in the quality evaluation and quality maintenance of poultry products. The research is conducted at Beltsville, Maryland, and at Athens, Georgia, in cooperation with the Georgia Experiment Station and through a research contract with the University of California, Davis, California.

The Federal scientific effort devoted to research in this area totals 6.0 professional man-years, about equally divided between objective measurement and evaluation of quality, and handling, packaging and storage.

Studies on the maintenance of quality during the off-farm handling, killing, dressing, eviscerating, cutting-up, chilling and packing of fresh poultry in the southeastern states (MQ 2-41) were terminated during this period.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

State stations maintain a continuing basic and applied research program on the market quality of poultry products. The regional effort in this area is conducted under NCM-7, Improving Market Quality and Utilization of Poultry Products. Personnel from eleven states and the United States

Department of Agriculture are cooperating on this regional project. Research emphasis is about equally divided between problems on eggs and on poultry meats.

With broilers, considerable effort is devoted to investigation of processing and storage conditions and their influence upon the shelf-life of raw poultry. Additional study is given factors which affect the quality of fresh, frozen and processed poultry meat in market channels. The quality, stability and acceptability of processed and pre-cooked poultry meat products is also studied with a view to development of more effective means for preserving quality. Other investigations seek to evaluate the effect of production practices on the composition and market quality of poultry meat.

Egg quality research is concerned with determination, evaluation and improvement of methods of controlling deterioration of shell eggs in market channels. Investigation of the effects of various treatments on the quality, nutritive value, stability and public health aspects of shell eggs and egg products involves study of: seasonal variation of egg quality; egg washing techniques; the biochemical changes which occur during marketing and storage; and certain causes of egg yolk discoloration which may relate to feeds used. The physical and chemical properties of egg shells are studied and attempts made to relate these shell properties to retention of the interior quality of the egg. Other research related to maintaining market quality involves design, construction and testing of experimental egg cleaners. Consumer studies attempt to relate consumer acceptance with egg grades and levels of interior quality.

The total research effort devoted to market quality research in poultry products is 20.4 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Quality measurement and evaluation of quality

1. Effect of Various Disease Syndromes on Wholesomeness of Market Poultry.

The primary emphasis in this study has been an attempt to artificially produce the severe type of air sacculitis most commonly encountered during official inspection in commercial poultry processing plants. This condition was not produced when the different groups of chicken fryers were inoculated with (a) Mycoplasma gallisepticum (S₆-PPLO) followed one week later with Escherichia coli or (b) with Pasturella multocida followed one week later by Infectious Bronchitis virus (IBV). The inoculation of fryers with (c) a Fowl pox vaccine followed three days later with M. gallisepticum intra-tracheally was also ineffective in producing air sacculitis.

Severe air sacculitis did develop when the following combinations of agents were used to inoculate chicken fryers: (1) a synovitis strain of PPLO when injected via the foot pad route; (2) Hemophilus gallinarum and the Roakin strain of Newcastle Disease Virus (NDV); (3) M. gallisepticum IBV, and E. coli (inoculations one week apart in that order); and (4) M. gallisepticum, IBV, and H. gallinarum (inoculations one week apart in that order). In the latter three trials, there was a tendency for lesions to resolve if birds were held long enough before slaughter. Thus, condemnation rates were higher for birds infected in the latter part of their growing period than for birds infected at an early age. Medication with antibiotics appeared to be of significant benefit only in the trial involving infection with M. gallisepticum, IBV, and H. gallinarum.

Artificially induced infections of turkeys of various age groups with M. gallisepticum (S₆-PPLO) affected younger birds most severely but at the market age of twenty weeks, condemnation rates of these birds were quite low indicating that they may overcome the infection prior to slaughter. A study of the action of the NTF strain of Mycoplasma in turkeys indicated that this agent was highly infectious. Turkeys infected at an early age suffered a much higher mortality rate than older groups after inoculation. At post mortem, birds that were in an active stage of the disease had such severe lesions that almost 100% were condemned.

The pathogenic action of Erysipelothrix insidiosa indicated that this agent was highly virulent, for both sixteen- and twenty-week old turkeys. Vaccinated birds were somewhat more resistant than non-vaccinated but did show appreciable mortality, particularly where they did not receive penicillin treatment. At termination, none of the remaining infected birds were condemned for unwholesomeness. Such carrier states were found twice as frequently in nontreated as in treated birds.

(MQ 3-22(c))

2. Assessing the Sanitary Quality of Egg Products. A comparison study of total viable aerobes, enterococci and coliforms in commercial egg products was completed. Under in-plant conditions, bacteriological examinations were made of liquid and dried egg products. In all liquid samples examined, coliforms were present in greater numbers than the enterococci. Significant correlations between the numbers of both enteric groups and the numbers of total viable aerobes were found in liquid whole egg but not in liquid yolk, whereas a significant relationship existed between the number of coliforms and the number of enterococci in all liquid products. All counts were found to be lower in liquid yolk than in liquid whole egg. This was thought to be due to the restricted contact of individual yolks with contaminated machine surfaces during the separation process. The total count of the yolk therefore did not appear to be a good index of sanitary practices.

Tests involving spray-dried whole egg and yolk products showed only minor changes in numbers of enterococci before and after drying whereas total viable aerobe and coliform counts were reduced. Subsequent storage of these products at 5° C for 8 months resulted in further decreases in coliforms and total aerobes and only a slight decrease in enterococci. Heat treatment of pan-dried egg white (130° F - 8 days) reduced all three counts to non-detectable levels. This project will be terminated.

(MQ 3-31)

3. Determination of Lighting for Proper Grading and Inspection of Poultry. Preliminary surveys to determine typical lighting conditions in processing plants have been made. "Daylight" type lighting equipment has been obtained. Installation and evaluation of lighting equipment is planned.

(MQ 3-52)

4. Methods to Evaluate Shelf-life of Eviscerated Poultry. Accuracy of Bacterial Sampling of Chickens. Bacterial counts of eviscerated chickens were determined in a commercial poultry processing plant to assess the variation in total aerobic bacteria among birds of the same lot and the variation among different areas of the same bird. Analysis of these data has been completed but evaluation and interpretation of the results are still in progress.

(Exploratory work -
Project Pending)

B. Quality maintenance in handling, packaging, and storage.

1. Chilling of Eviscerated Broilers. A comparison of three methods of chilling eviscerated broilers, i.e., (1) in still air at 40° F; (2) in an agitated ice plus water mixture; (3) or in agitated chilled water, was made. Ice plus water chilling caused a significant percentage reduction of bacteria per cm² of skin as compared to the chilled water process. Chilling in 40° F air caused significant increases in bacterial counts. Both wet chilling methods resulted in increases in carcass weight whereas air chilling resulted in slight weight loss. No organoleptic differences were detected in either broth or meat from carcasses chilled by the three methods.

(MQ 2-41)

2. Bruising of Broilers. A study on factors affecting bruising of broilers indicated that the manner of loading live birds into coops on trucks has a significant effect on the incidence of bruising of broilers after slaughtering. A significantly lower percentage of birds with bruises or discolorations was found in the middle layer of coops than either the top (ninth) or bottom layer. Aluminum coops and coops whose openings were padded with foam rubber, also reduced the number of bruised birds.

(MQ 2-41)

3. Quality Retention of Eviscerated Poultry as Related to Method of Slaughter. The rate of blood loss and amount of blood retained by cut-up chickens was determined when birds were slaughtered, using the following methods: (1) electric stun and outside cut; (2) brain stick and outside cut; (3) skull puncture and outside cut; (4) carbon dioxide immobilization and outside cut; (5) Kosher cut; and (6) outside cut. Blood loss was determined at 30 seconds, 90 seconds, 3 minutes, and 3-5 minutes after slaughter. The blood remaining in the cut-up parts was determined by assaying the radioactivity of the part resulting from ante-mortem injection of radioactive iodinated serum albumin (I^{131}). The parts assayed were breast, wings, thighs, drumsticks, kidneys and Bursa of Fabricius, back, neck, liver, heart, gizzard, head, feet, spleen, and offal. The data are presently being analysed.

(MQ 2-81)

4. Changes in Eggs During Cold Storage. Determinations were made of inorganic phosphorus, reactive NH_2 , and glucosamine in eggs stored up to nine months. No important differences were found. It was determined that the thinning of egg white was not related to splitting of a hyaluronic acid-protein complex.

(E25-AMS-5(a))

5. Oxidative Deterioration of Cooked Poultry. A mixture of polyphosphates has been found to be effective in delaying oxidative deterioration in commercially processed and cooked fryer chickens. After storage of one week at $40^\circ F$, phosphate-treated chicken meat showed no, or very slight, off-odor and a thiobarbituric acid (TBA) value of about 1, whereas untreated control chickens had a slightly to moderately strong off-odor and a TBA value of about 6.

(Exploratory Work -- Project Pending)

6. Radiation Pasteurization of Eviscerated Poultry. Fresh and frozen tray-packed cut-up fryer chickens were irradiated with a cobalt-60 source with 0.0, 0.1, 0.3, and 0.5 Mrads. Irradiation at the three levels conferred additional days of shelf-life on both fresh and frozen birds when they were subsequently stored at $34^\circ F$ and at $40^\circ F$. There appeared to be no significant difference in shelf-life between fresh birds receiving the three levels, nor between those stored at 34° or $40^\circ F$. A shelf-life of 23 to 25 days was found at all three levels as compared to 11 days for the unirradiated controls. Shelf-life extension of birds irradiated in the frozen condition was not as great (4 to 5 days extension) at the 0.1 Mrad level as it was at the two higher levels. Data from panel evaluations for off-odor in both the raw and cooked chickens are being analyzed. A study of the development of oxidative deterioration in chickens which had been irradiated in the frozen

condition at 0.1 and 0.5 Mrad level and subsequently stored at 40° F up to 27 days and at -15° F up to 14 weeks was also carried out employing panel evaluations for off-odor and the TBA test. Cooked samples of the irradiated frozen chicken stored at 40° F were also tested. Analyses of these data are in progress.

(Exploratory Work)

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Quality Measurement and Evaluation of Quality

Tarver, F. R., Jr., and K. N. May. 1963. Effect of slaughter technique, immersion scald, and refrigerated storage on bacterial counts of poultry air sacs. Poultry Sci. 42:1141-1145.

(MQ 3-22(c))

Tarver, F. R., Jr., and K. N. May. 1963. Kinds of aerobic bacteria in air sacs of processed poultry. Poultry Sci. 42:1459-1460.

(MQ 3-22(c))

Quality Maintenance in Handling and Packaging

Cox, C. J. and K. N. May. 1964. Factors affecting bruising of commercial broilers. Ga. Agr. Expt. Sta. Technical Bulletin NS 35.

(MQ 2-41)

Kotula, A. W. and J. A. Kinner. 1964. Airborne microorganisms in broiler processing plants. Applied Microbiology 12(3):179-184.

(MQ 2-10)

Kotula, A. W. and A. J. Mercuri. 1964. Food preservation by gamma irradiation - a review. The Maryland Poultryman, July, pp. 1-5.

(Exploratory work -
project pending)

May, K. N. 1964. What happened to the "boom" in frozen poultry. Broiler Business, June. p. 16

(MQ 2-41)

May, K. N. and R. L. Saffle. 1964. Quality of ice-packed and frozen chicken. 2. Taste panel evaluations. Poultry Science, 43(4): 1044-51.

(MQ 2-41)

Rogers, P. D. and K. N. May. 1963. Cooling of eviscerated broilers in chilled water. Ga. Agr. Expt. Sta. Technical Bulletin 34.

(MQ 2-41)

Walters, R. E. and K. N. May. 1963. Thermal conductivity and density of chicken breast muscle and skin. Food Technol. 17:130-133.

(MQ 2-41)

Winawer, H. H. and K. N. May. 1964. Quality of ice-packed and frozen chicken. 1. A consumer preference study. Poultry Sci. 43(4):1031-1035.

(MQ 2-41)

Stokes, D. E., A. W. Kotula, A. J. Mercuri, and F. K. Buxton. 1964. Evaluation of specified shrink films for prepackaging frozen cut-up chickens at processing plant. USDA, AMS, Marketing Research Report No. 662, 15 pp.

(Exploratory Work -
project pending)

MARKETING FACILITIES, EQUIPMENT, METHODS,
 CONSUMER PACKAGES AND SHIPPING CONTAINERS ^{1/}
 Transportation and Facilities Research Division, ARS

Problem: A continuing increase in the production of poultry and eggs in the United States and, in recent years, a marked increase in total consumption of poultry has resulted in significant changes in marketing methods and requirements for these products. These changes have had an impact on poultry processing and egg grading and packing plant facilities and equipment requirements as well as in operating methods. In order to improve operating efficiency in existing facilities and provide guidelines for plant expansion or for new facilities, more efficient work methods, plant layouts, devices, and equipment, and improved facility designs are needed for commercial handling, processing, grading, and packing of poultry and eggs.

It costs about 8 billion dollars a year to package food products, but without shipping containers and various other types of packages it would be impossible to move farm products efficiently from the widely dispersed areas of production through our complex marketing system to millions of consumers. New or improved packages and containers must be developed and evaluated to do this job more effectively. In protecting, distributing and selling perishable agricultural commodities, packages and containers must respond to a number of marketing system changes.

^{1/} The work described here is part of an overall program aimed at improving market facilities and market operations. As agricultural commodities flow through marketing channels they converge with similar products, for example, meat, poultry, fish and dairy products are often handled by the same wholesaler and reach consumers through the meat and produce department of retail stores. Because of this situation, improvements in the overall marketing process can bring about benefits that affect several commodities simultaneously. The component costs of marketing have been rising rapidly and would have risen more if the results of this type of research had not been available. In the marketing of food commodities in 1963, at least \$30 billion (75% of the total food marketing bill) were expended on marketing operations that are directly affected by the research covered in the overall program. The overall program includes (1) terminal wholesale marketing planning, (2) preliminary and followup work in terminal market areas, and (3) production area and independent marketing facilities such as that described here. Terminal wholesale market planning was conducted in 7 major cities last year. Production area and independent market facilities planning involved 41 studies. For additional information see "A Summary of Current Program and Preliminary Report of Progress" dated September 30, 1964, by the Transportation and Facilities Research Division, ARS, USDA.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program devoted to planning marketing facilities in which application is made of engineering, economic, and marketing principles. This work is concerned with structures, equipment, containers, devices, work methods, and operating methods used in marketing and transporting farm and food products from farms to consumers. The functions to which these physical elements, handling methods, and labor relate include essentially all marketing operations especially those directly applicable to the commodities in the physical sense such as assembling, preparing for market, processing, packaging, precooling, loading, transporting, unloading, storing, warehousing, and wholesale and retail distribution. The part of the program pertaining to poultry involves engineers and marketing research analysts engaged in both basic and applied research to develop more efficient work methods, techniques, operating procedures, devices, and equipment and to design improved facilities for the handling and preparing of poultry, eggs, and egg products for market. Research on chicken-class poultry processing operations is carried on in commercial poultry processing plants in the Southeast by Department personnel at Athens, Ga., in cooperation with the Georgia Agricultural Experiment Stations. Contract research on shell egg cleaning techniques and equipment conducted by the University of California at Davis, Calif., was completed during the report year. Contract research to determine the optimum production rates in chicken processing plants for the eviscerating and federal post-mortem inspection operations was initiated during the report year and is being conducted by the American Scientific Corporation at Alexandria, Va., and in selected poultry processing plants on the Delmarva Peninsula. Research on turkey-class poultry processing, egg products processing, and cleaning, grading, and packing shell eggs is carried on in West Coast turkey processing and in egg grading and packing plants in cooperation with the California Agricultural Experiment Stations at Davis, Calif.

The Federal effort devoted to research and development work in this area during the fiscal year 1964 totaled 7.1 professional man-years: 3.6 man-years (including 0.6 man-year of contract work) on poultry, 2.3 man-years (including 0.6 man-year of contract work) on shell eggs and egg products, 0.1 on consumer packages and shipping containers, and 1.1 man-year on program leadership.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Methods, Equipment, and Facilities for Grading and Packing Eggs.

This research concluded in Hyattsville during the report year was directed toward reducing egg marketing costs through engineering and marketing research involving a systems analysis of existing work methods, equipment, and facilities for grading and packing eggs, and the development of improvements. A report of the results of this study has been compiled in a manuscript, "Evaluation of Mechanized Egg Grading and Packing Equipment" which is being reviewed and edited for publication. It will provide guidelines for egg grading plant operators in selecting the degree and combinations of mechanized egg grading and packing equipment that will yield the greatest output per worker and equipment dollar at specified production and product quality levels.

B. Improved Designs for Wholesale Poultry and Egg Distributors' Warehouses

The results of this research, reported last year, were published in MRR 630, "Multiple Occupancy Warehouses for Poultry and Egg Wholesalers, Improved Designs." In addition to furnishing builders and poultry and egg wholesalers with layout, operating, and facility guidelines for multiple-occupancy type warehouses, it is also being used by egg grading and packing plant operators seeking information and assistance for the construction and operation of small egg breaking rooms required for processing undergrade shell eggs.

C. Improved Methods, Techniques and Equipment for Cleaning Eggs

This research conducted at Davis, Calif., involves the development of techniques and equipment that will do an effective in-line job of cleaning shell eggs with minimum quality deterioration and breakage, and that will minimize labor requirements. The basic studies of typical spoilage organisms to determine time and temperature conditions necessary for safe, commercial egg washing, the testing of commercial washers, and the design and the construction and testing of an experimental washer were carried on under contract with the University of California. Preliminary results of this basic work were reported in a technical paper, "How You Can Wash Eggs Cleaner" by A. W. Brant, University of California. A completed manuscript, now being reviewed and edited, reports: (1) The bacteriological, chemical and physical requirements for commercial egg cleaning; and (2) the laboratory test results of the experimental cleaner showing that it removed stain and dirt from 90 percent of the soiled eggs washed, while commercial equipment cleaned only 21.7 percent, and that it practically eliminated the hazard of spoilage due to washing.

The field tests of the experimental cleaner, conducted by Department personnel, and comparisons of the results with those from commercial cleaners have been completed. Preliminary evaluation of the data indicates that almost the same safe cleaning effectiveness was maintained when the experimental equipment was operated under commercial conditions as that achieved under laboratory conditions. The findings on these tests are now being assembled for publication as a research report.

D. Improved Layouts and Engineering Designs for Egg Grading and Packing Plants

This long-term research, carried on at Davis, Calif., is directed toward the development of improved designs and layouts for small egg grading and packing plants, involving preparation of engineering drawings and development of building specifications. As large-scale commercial egg production units in the U.S. have increased in size and number, there has developed a need for structural designs and layouts for small egg grading and packing plants located at or near the source of production. These units will be designed to handle the output from one, two, or three large commercial flocks. Many of these plants are now located in buildings that were not designed for this type operation. As a direct result, the economic advantages that large lots of uniformly fine quality eggs offer through mechanized handling, are frequently lost, product quality is exposed to the hazards of improper environment, and labor and equipment utilization is poor.

Observations have been made in a number of plants typical of this category to determine production capacity requirements and desirable features of existing facilities. Because of urgent requests for assistance from industry, special emphasis is first being placed on the design and layout requirements for a small egg breaking room area required in these plants. A technical paper on egg products facility requirements was presented to industry representatives. An interim report will be prepared on the design and layout recommendations for small egg breaking operations.

Case study plants for overall evaluation will be selected and discussed with supervisory engineers and marketing specialists as structural designs are developed.

E. Improved Methods and Equipment for Eviscerating Chickens

This long-term research program, at Athens, Ga., involved the development of methods, operating procedures, and equipment for eviscerating chickens. Completion of this research involved publication of Marketing Research Report No. 651, "An Experimental Dual Track Conveyor System for Processing Poultry." It showed that through the use of the combination power and free conveying principle together with an adaptation of mechanical line dividers, electrically operated carrier diverters, and convergers and temporary storage conveyor loops (all devised during the study); chickens can be routed through processing plant operations on individual carriers at changes in processing rates with less labor and a reduction in production "bottlenecks."

F. Improved Methods and Equipment for Cutting Up and Packing Chickens

This research, conducted at Athens, Ga., involves the study of the methods, equipment, and facilities for further processing chicken meat--cutting up whole ready-to-cook chicken and packing the output by individual parts, combination of parts, or as whole cut-up chicken--a merchandizing trend that currently involves about 1 billion pounds of chickens annually. Initial research in this area; carried out under research contract with the Gordon Johnson Equipment Co., Kansas City, Mo., in cooperation with the J. D. Jewell Co., Gainesville, Ga.; sought to reduce the relatively high labor requirements and costly losses sustained in frequently packing overweight in containers marked with the exact weight, by developing an accurate, rapid, mechanical weighing and selecting device for chicken parts. The results are now in manuscript form. They describe the development and testing of an experimental parts sizing and sorting machine that mechanizes the accurate selection of the last chicken part required to fill a container to an exact weight. Test results showed that it can reduce the amount of meat "given away" by $\frac{1}{2}$ ounce per 1-pound package and lower the packing labor requirements by 35 percent. The manuscript will be published as a marketing research report.

G. Improved Designs for Chicken Processing Plants

This long-term research, carried on in Athens, Ga., involves the development of guidelines for improved plant designs and layouts, furnishing maximum efficiency at minimum cost for new construction or plant modernization. The rapid growth of the poultry industry has resulted in building additions and frequently makeshift construction without sufficient regard to or utilization of space. New structures have frequently included errors in building design and plant layout because of an absence of basic guidelines. Designs and layouts for the basic plant work and service areas have been developed from a composite source of data--recommendations from recently completed research in the packing, eviscerating, and further processing areas, from review of approved blueprints of official U.S.D.A. plants, and from layout and design studies of plants that demonstrate effective utilization of plant space and personnel during current operations. A manuscript draft covering overall layout requirements, the layout of each work area, and development of plant designs directed toward efficient operation and economic expansion has been completed.

H. Improved Methods, Equipment and Facilities for Improving Chicken Processing Plant Efficiency Through Balance of Inspection and Eviscerating Operations

This study is part of a long-term research program carried out under a contract with the American Scientific Corporation at Alexandria, Va. Field studies are being conducted in selected chicken processing plants on the Delmarva Peninsula. It is directed toward increasing chicken processing plant efficiency and reducing the Department's costs in carrying out mandatory inspection in official U.S.D.A. plants. Research findings reported

in MRR 549, "Methods and Equipment for Eviscerating Chickens" showed that maximum eviscerating crew labor utilization was reached at different production levels for certain combinations of equipment and crew sizes and composition. Extending production beyond these optimum rates not only decreases the output per plant worker, but frequently requires additional inspection personnel that is not fully utilized. Through the application of findings reported in MRR 549, and by conducting similar studies of inspection operations, analyzing production line speeds, types of equipment and processing crew makeup, guidelines for balanced operations will be developed for specified production levels while attaining maximum equipment and floor space utilization and maintaining an acceptable level of workmanship.

The first progress report covering the evaluation of the inspection operation in plants using specified conveying equipment, while operating at 1,000 to 3,000 chickens per hour, has been submitted by the contractor for review and acceptance. Optimum processing rates, inspection-eviscerating crew makeup and equipment combinations are predicted for specified production levels. After similar predictions are developed for plants operating in the 3,000 to 5,500 and 5,500 to 8,000 bird-hour rates, all predictions will be tested, adjusted as necessary, and a report prepared to provide guidelines for optimum crew (inspection and eviscerating) and equipment combinations.

I. Improved Methods and Equipment for Handling Live Chickens by Commercial Processing Plants

This research, carried on at Athens, Ga., involves studies of live chicken handling operations to determine the methods and equipment that cause the relatively high rate of bruising of live chickens prior to slaughter and to develop improvements that will minimize this costly damage that frequently results in "down grade" penalties to as much as 15 percent of a flock. In addition, efforts will be directed toward modernizing the equipment and methods so as to reduce the relatively high labor requirements and hazards of flock contamination. Preliminary studies of live handling and unpublished research findings indicate that broiler-class chickens respond favorably to the quieting effect of some types of external stimuli and could be useful in developing improved cooping methods and equipment as an aid to reducing "field" bruising. These possibilities will be explored as the analysis of existing methods and equipment is carried out. Interim reports will be prepared and published as the research progresses.

J. Improved Methods and Facilities for Chilling, Weighing, and Packing Turkeys

This research, carried on at Davis, Calif., is part of a long-term research program dealing with the development of improved methods, equipment and facilities for chilling, weighing, and packing turkeys and is similar to the research carried on in chicken processing plants at Athens, Ga.

Because of the relatively long time required to chill turkeys properly after evisceration, and the difference between this rate and the packing rate, the packing area in most turkey processing plants forms a "bottleneck" in product flow and is a direct cause of poor labor, equipment and space utilization. Further, operating procedures and equipment frequently permit sufficient "weepage" after weighing and packing so as to bring about short weight and unsightly wrapping stains. Because of the low projected return for their efforts, major equipment companies have not actively engaged in equipment development programs in this plant area.

This research is directed toward reducing the relatively high labor requirements for placing whole eviscerated turkey carcasses into their ultimate consumer package, minimizing weepage problems, and synchronizing these operations with the preparatory steps that precede them. Based on evaluation of existing operations through method analysis, improvements are being developed. A weighing-bagging station was designed and shop tested. Preliminary results indicate that it has the potential for reducing the physical effort required for this task and also reducing the crew size by two or more workers for packing turkeys on a 1,200 bird-per-hour production line. This equipment is now in the process of being field tested under commercial operating conditions. A report will be prepared setting forth the results of the tests, and will make comparisons with existing operations. Methods for reducing or eliminating other bottlenecks in the packing area are being considered and evaluated.

K. Improved Methods, Equipment and Facilities for Preparing Turkey Specialty Items

This research is part of a long-term program carried on at Davis, Calif. It is directed toward the development of guidelines for improved methods, equipment, and facilities for preparing many of the specialty items currently being marketed from cut-up ready-to-cook whole turkey. In recent years the trend toward preparing turkey specialty items in turkey processing plants designed only for slaughter, defeathering, evisceration and chilling has increased rapidly. Cut-up operations, deboning, preparing turkey logs and other specialties have frequently been carried on in overcrowded makeshift facilities, involving methods high in labor requirements and employing equipment of questionable value. This has resulted in production bottlenecks, hazardous to quality, and relatively high processing costs. Through process and method analysis, study of flow patterns and materials handling needs, improvements in work area layouts will be developed, and improved methods will be developed, tested, and evaluated in order to provide an efficient economic preparation of product beyond the whole ready-to-cook turkey stage. Through industry contacts, access to typical plants has been established and a number of case study plants have been selected. As improvements are developed and tested, appropriate reports will be prepared.

L. Consumer Packages and Shipping Containers

Five types of clear shrinkable films were evaluated as wraps for cut-up chickens prepackaged and frozen in a Georgia processing plant. The prepackaged frozen birds were trucked to a Philadelphia warehouse and later put on sale in Wilmington, Del., food stores.

The films evaluated were: (1) 1/2-mil cast polyvinyl chloride; (2) 1-mil extruded polyvinyl chloride; (3) 1-mil irradiated polyethylene; (4) 1-mil polyethylene; and (5) 1-mil polypropylene.

Upon arrival of the packages at the retail stores, the 1/2-mil film showed substantially more tearing than the 1-mil films; among the four 1-mil films, differences in the incidence of tearing were not significant. All five films held up well while on display.

Although this was the first time that frozen cut-up chickens had been sold on a commercial scale in Wilmington, the shoppers bought more than had been expected, and as time went on, sales increased relative to sales of fresh chickens. The shoppers appeared to find no differences among the five test films, purchasing one about as readily as another.

PUBLICATIONS -- USDA COOPERATIVE PROGRAMS

Improved Methods, Techniques and Equipment for Cleaning Eggs

Brant, A. W. 1964. How You Can Wash Eggs Cleaner. A technical paper presented at the Fact Finding Conference, Institute of American Poultry Industries, Kansas City, Mo. Feb. 1964.

Improved Methods and Equipment for Eviscerating Chickens

Walters, R. E., Childs, R. E., White, H. D. 1964. An Experimental Dual Track Conveyor System for Processing Poultry. Mktg. Res. Rpt. No. 651. 24 pp.

Improved Designs for Wholesale Poultry and Egg Distributors' Warehouses

Hamann, J. A. 1963. Increased Efficiency for Poultry and Egg Wholesalers' Operations Through Plant Design Planning. A technical paper presented at the Annual North Central States Poultry and Egg Exposition and Conference, Omaha, Nebr. December, 1963.

Hamann, J. A., Forbus, W. R. 1964. Multiple Occupancy Warehouses for Poultry and Egg Wholesalers, Improved Designs. Mktg. Res. Rpt. 630. 39 pp.

Methods Equipment and Facilities for Grading and Packing Eggs

Hamann, J. A. 1964. Mechanized Egg Grading and Packing Lines. A technical paper presented at the Annual Meeting of the Poultry Science Association, University of Minn., Minneapolis, Minn. August 1964.

Improved Methods and Equipment for Cutting Up and Packing Chickens

Hamann, J. A. 1964. Mechanized Packing of Chicken Parts. A research report presented on WMAL and WRC television networks. January 1964.

Improved Layouts and Engineering Designs for Egg Grading and Packing Plants

Robbins, R. O. 1964. Facility and Equipment Needs and Layout Planning for Egg Breaking Rooms. A technical paper presented at the University of California Egg Products Workshop, Davis, California. June 1964.

Consumer Packages and Shipping Containers

Stokes, Donald R., Kotula, Anthony W., Mercuri, Arthur J., and Buxton, Freeman K. 1964. Evaluation of Specified Shrink Films for Pre-packaging Frozen Cut-up Chickens at Processing Plant. Marketing Research Report 662, pp. 15, July 1964.

COOPERATIVE MARKETING Farmer Cooperative Service

Problem: Farmers are expanding their use of cooperative marketing. There are constant changes in transportation, processing, and distribution technology, and in market organization and practices, and changes on the farm itself. In view of these developments, farmer cooperatives and other marketing firms require research results to perform both efficiently and effectively. Such research can assist farmers to maintain and strengthen their bargaining power, increase efficiency, and meet the quality, quantity, and service needs of today's food and fiber marketplace.

Cooperative marketing is a major way for farmers to get maximum returns from their products in the current and rapidly changing market. Farmers own and control cooperatives specifically to increase their income from crops and livestock. Gains are not automatic, however. Cooperatives must plan, develop, and actually manage the specific marketing program and services that will yield the most for their members. Marketing cooperatives must know what the market demands. They must be able to compute the probable cost of different ways of serving the market. They must understand the possibility of major economies in a well coordinated joint sales program, and understand the methods and potentials of bargaining. Management must achieve minimum costs through improved organization, good use of existing plant and personnel, and the selection and use of new equipment and methods.

USDA PROGRAM

The Department conducts a continuing long-range program of basic and applied research and technical assistance on problems of marketing farm products cooperatively. Studies are made on the organization, operation, and role of farmer cooperatives in marketing. While most of the research is done directly with cooperatives, the results are generally of benefit to other marketing firms. The work is centered in Washington, D.C. Many of the studies, however, are done in cooperation with various State experiment stations, extension services, and departments of agriculture.

Federal professional man-years devoted to research in this area totaled 23.3. Of this number, 1.0 was devoted to cooperative marketing of citrus, 2.7 to cotton, 4.5 to dairy, 1.2 to deciduous fruit, 2.2 to grain, 3.9 to livestock and wool, 1.3 to oilseeds and peanuts, 1.0 to potatoes, 3.5 to poultry, 0.1 to rice, 0.6 to tobacco, and 1.3 to vegetables.

Research also is conducted under contract with land-grant colleges, universities, cooperatives, and private research organizations. During the period of this report, contract research was performed by universities and colleges in Florida, Iowa, Louisiana, Montana, North Dakota, and West Virginia, and by one private research company.

STATE EXPERIMENT STATIONS PROGRAM

The State stations maintain a very broad research program in commodity marketing, the findings of which are valuable to cooperatives and to other marketing firms. There are at this time nine projects in eight States that deal specifically with cooperative marketing. Five projects are commodity oriented and deal with grain, tobacco, milk, livestock, and fruits and vegetables. These projects seek to find out how cooperatives are adjusting or might better adjust to changes in market structure and marketing practices. In some instances researchers are studying the success and failure of cooperatives and the organizational structure. One study of the history of major cooperative marketing associations in the State will be published as a book and will undoubtedly receive nationwide attention.

Because of the growing interest in the role of cooperatives in market structure, one State recently initiated a major project in this area. The project leader views cooperative enterprises as a structural dimension of farm markets. The objectives and operating procedures of cooperatives will be studied to see if they have a unique impact upon market conduct and performance. If so, this may have significant implications for Government policies and programs.

The total research effort on cooperative marketing in the eight States is 3.4 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Coordination of marketing

Farmers and their cooperatives need to adapt their marketing methods to the requirements of large-scale buyers, mass merchandising, and other changed conditions. In many cases the coordination of marketing of a number of cooperatives, marketing the produce of hundreds or thousands of farmers, will satisfy these needs and improve returns to farmers. Such coordination may be accomplished by establishment of joint sales agencies or by other methods. Research to determine the problems and needs, and to develop guides for adopting new practices, included work with several commodities.

Poultry. A study was completed providing plans for a coordinated marketing effort by cooperatives also engaged in rendering production services to poultrymen. Studies were undertaken to assist cooperatives in considering merger, and developing plans for such merger or consolidation.

B. Improving cooperative sales and distribution methods

In many commodity fields, wholesale and retail marketing practices have changed so much that sales and distribution methods need to be restudied from the farm level forward. Research on these problems included work in several commodities.

Poultry. Examination continued of potential ways to increase producer returns for fowl. Findings indicated that (1) fowl has suffered a drastic loss of value at farm level, (2) the further processing industry absorbs most of the fowl but lacks competition, (3) prices realized by producers have been low in relation to the utilization value of fowl, (4) more competition, new marketing, and new merchandising methods are needed, (5) producer-owned facilities could be established to provide such competition and improved practices.

C. Potentials in cooperative marketing

In several commodity areas an appraisal is needed of the present and potential role of cooperative marketing. Current information on cooperative operations can be related to production and marketing conditions. This research will yield suggestions about cooperative operations and services, and provide current data needed by cooperative leaders and others for planning and implementing cooperative marketing programs.

Poultry. An appraisal was initiated of the functions and potentials of broiler auctions. This research will determine the origin, history, and present economic condition of live broiler auctions. The influence of auctions on broiler pricing will be analyzed, including study of areas where auctions developed and where they did not. Factors leading to broiler auction success or failure will be studied. Findings of this study should permit appraisal of the potential role of broiler auctions, and recommendations for improving broiler auctions.

D. Pooling and pricing

Pooling principles and procedures must be periodically examined as to their effect on equity among members and efficiency in marketing. In some commodities, pooling has not come into widespread use, and the use of pooling needs to be studied and its application considered.

Poultry. Study continued of pooling practices of cooperatives in marketing eggs. A report is being prepared, including suggestions for the adoption of sound and equitable pooling payment practices.

E. Improving operating methods in processing and storage

Studies were underway in several commodity fields to examine new methods, equipment, facilities, and structures for efficient and safe processing and storage of agricultural products by cooperatives.

Poultry. Policies of poultry cooperatives were studied to identify ways such cooperatives might increase their volume by attracting large producers, thus reducing operating costs.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Scanlan, J. J. 1964. Poultry Co-ops Work for Top Efficiency. News for Farmer Cooperatives (Jan.).

MARKET POTENTIALS FOR NEW PRODUCTS AND USES
Marketing Economics Division, ERS

Problem: Increased emphasis should be placed on new products and new uses because of their importance in expanding markets and maintaining a high rate of economic growth. Agricultural producers and processors need to take maximum advantage of the opportunities offered with respect to additional outlets for surplus supplies, increased returns, lowered costs, and improved competitive positions relative to non-agricultural products. Continuing evaluations are needed of the commercial feasibility and market potentials of new or improved agricultural products, by-products, and products from new crops in food, feed, and industrial uses; of the economic feasibility of developing new uses and establishing new crops, including appraisal of their impact on present markets; and of the economic and technical requirements of end-uses. Such evaluation will provide a sound economic base for decisions on commercial developments, as well as information to guide further utilization research by physical scientists.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving agricultural economists and personnel with dual economic and technical training engaged in research to bridge the gap between laboratory developments and commercial adoption to assist producers to realize more rapidly and more fully benefits of lowered costs, increased returns, and expanded markets that new products and new uses can afford. Research is carried on in industrial and food uses at Washington, D.C., and six field offices -- agricultural economists are located at each of the four Utilization Research and Development Divisions, New Orleans, Louisiana; Albany, California; Philadelphia, Pennsylvania; and Peoria, Illinois. Economists are also stationed at the Hawaii Agricultural Experiment Station, Honolulu, Hawaii, and at the Department of Agricultural Economics, Clemson University, Clemson, South Carolina.

Research is conducted on animal products, cotton, grain and forages, oilseeds, horticultural crops, new crops, and on impacts of technological innovations. Cooperative research is conducted with the Hawaii Agricultural Experiment Station on Kona coffee and Hawaiian fruits and vegetables, with the Pennsylvania Agricultural Experiment Station on maple products, with the Louisiana Agricultural Experiment Station on a new sweetpotato product, and with Clemson University on market potentials for modified milk. Producer groups, such as the Louisiana Sweetpotato Commission and the Michigan Apple Commission, contribute to studies of potentials of new products pertaining to their area of interest.

The Federal scientific effort devoted to research in this area totals about 19.9 man-years. Commodity-wise, 4.7 man-years are currently devoted to animal products; 3.1 to grains; 2.6 to oilseeds and sugar; 3.9 to horticultural crops; and 5.6 to other research, principally new crops and impact of technological innovations.

PROGRAM OF STATE EXPERIMENT STATIONS

Little, if any, research in economics is carried out in this area by State agricultural experiment station personnel. Much research is being conducted on the development of improved products and uses, but it is in the area of technology.

PROGRESS -- USDA AND COOPERATIVE PROGRAM

1. Egg Processing Costs. Data have been collected and analysis begun on comparative costs of preparing and marketing egg products by freezing and drying. Costs, together with other information on the various properties of eggs processed in various forms, will provide a basis for most efficient use of egg products in particular uses.
2. Fats in Feeds. Brief reviews are made from time to time to check conditions and progress in markets previously researched but which are of continuing importance. A change in the source of data collected by Census on consumption of fats in feeds reveals that this market for fats is much larger than had been reported. Revised figures upped consumption of inedible tallow and grease in feeds by 76 percent in the first quarter of 1964 and indicate the use of 800 million pounds this year. A study of market potentials for fats in feeds, reported in MRR 498 (Sept. 1961), projected that fat use would reach to an annual rate of 1.4 billion pounds by 1970. Reported use in subsequent years did not show an increase over the 1961 rate even though all research evidence indicated otherwise. By collecting data from renderers instead of feed manufacturers, Census is now obtaining a more accurate coverage of tallow and grease disposition. Other fats still not reported, such as poultry oil and hydrolyzed foots, would swell total use by almost another 100 million pounds a year. The feed market now outranks the soap market as an outlet for fats and oils in both price and volume.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

MERCHANDISING AND PROMOTION PRACTICES
Marketing Economics Division, ERS

Problem: Problems of selling efficiency, consumer acceptance, orderly distribution, and coordination have grown in scope and complexity as major changes have occurred in the production, processing, and distribution of farm products. Because of the wide array of products made available to consumers, through self-service retailing, as well as other factors, merchandising, promotion, the control of product distribution and movement, and management decision-making have increased in importance as basic and essential functions in the marketing of farm products.

Because of self-service not only must a product offered in today's supermarket be its own salesman but also it must compete directly or indirectly with thousands of other items for consumers expenditures. Substantial sums are being spent by farm groups, food processors, and retailers in merchandising and promotional efforts.

Information is needed by producer groups as well as distributors at other levels in the marketing channels to determine the effectiveness of alternative promotion, merchandising and advertising techniques, levels of promotional intensity necessary for maximum sales response and the characteristics of products lending themselves to promotional stimuli. There is a basic need for development of principles or guidelines broadly applicable to agricultural commodities and which may be used in developing and strengthening commodity promotion. More effective merchandising of farm products is also required if demand is to be influenced and greater consumer acceptance gained for farm products. Sales of individual products have become increasingly dependent on in-store merchandising that will attract consumers and influence purchases. This is particularly true for many farm commodities which are not pre-sold through intensive advertising.

Because of increased complexity of operation, firms processing and distributing farm products need information which will assist in improving management efficiency. Smaller firms and particularly those operated by producers often do not have the resources or experience necessary to develop the information or techniques necessary for more efficient operations.

USDA AND COOPERATIVE PROGRAMS

The Department has a continuing long-term program of research in merchandising, management analysis, product distribution, and promotion evaluation to provide information which can be used by producers, handlers and distributors in strengthening and expanding markets for farm products. The merchandising research program is designed to quantitatively evaluate the impact of selected selling practices and price policies on the demand for agricultural products. Specific studies have as their objectives the development

of income-expenditure elasticities and measurement of other factors influencing demand, determination of consumer and market profiles, and evaluation of alternative merchandising techniques such as packaging, display, pricing, featuring and product variation on consumer purchases.

Research relating to promotion and advertising includes studies to: determine organizational structure and procedures of commodity promotion groups for optimum control, coordination and conduct of their program; measure levels of advertising and promotional intensity required to influence sales, evaluate relative effectiveness of alternative promotional appeals, themes, and techniques, and develop principles applicable to the promotion of farm products.

Studies of product distribution, such as availability, movement of products into consumption, and profiles of markets and consumers, provide information by which sound advertising, merchandising and management decisions can be made. In addition, management type studies are conducted to provide techniques and procedures which can be used to coordinate the diverse marketing functions and improve efficiency of firms distributing farm products. Most merchandising and promotion studies are conducted in close cooperation with producer or industry groups, food wholesalers, and retailers. Industry groups giving direct financial support to research during the year include, the American Dairy Association, the Florida Citrus Commission, and Florists' Telegraph Delivery Association.

During fiscal 1964, approximately 14.5 professional man-years were directed to the area of merchandising and promotion. Of this total, 2.0 were devoted to dairy; .3 to beef; .3 to poultry; 1.1 to grains and forage; 3.5 to citrus and subtropical fruits; 1.1 to deciduous fruits and tree nuts; 2.0 to flowers, ornamentals and shade trees; and 5.2 to cross-commodities.

The research effort is centered in Washington, D. C., with professional employees stationed at State Experiment Stations in Washington and Indiana. Cooperative studies are being conducted with the following State Experiment Stations: Arizona, Indiana, Ohio, and Washington. Many studies involve data collection on a national basis while others involve case studies, and controlled experiments in selected locations.

PROGRAM OF STATE EXPERIMENT STATIONS

Much of the research at the State agricultural experiment stations in the area of merchandising and promotion is carried out in connection with specific commodities and thus reported under those headings. That reported here is problem-oriented and only incidentally commodity-oriented. Thus, to get the total effort in this area one would need to add that reported under the specific commodity sections of this report.

Consumer acceptance, preference and attitude studies represent an on-going phase of the State program. Current work deals with food and fiber items

as well as nursery products. This research is undertaken for the purpose of market test and development and, thus, is closely allied with the product development phases of the Stations program. In the year reported, this research totaled 11.4 professional man-years.

Research on consumer motivation and decision-making is underway at 15 State stations. These studies are concerned with type and amount of influence resulting from food promotion and consumer information programs, with factors affecting food purchase decisions, and with consumer behavior in the market place. Limited work is also underway on improved consumer grades for agricultural products. This phase of the market development research totals 17.7 professional man-years.

Research Example--Children's Role in Influencing Food Purchases, Miss. Project 1238.

While children's influence upon family food practices is readily accepted, there is little knowledge about the nature and extent of their role. The Mississippi Agricultural Experiment Station reports that nine- and ten-year-olds are like their elders in that they know somewhat more about food needs than they put into practice. The usual reason for not eating needed foods was that they were not provided by the family. The children studied learned about food from many sources, especially those involving social activity. Market promotion schemes (labels, coupons, and premiums) seemed to have little effect upon them. They reported that requests of parents to buy a food were usually granted, and that all types of food were requested--meat, vegetables, sweets, and soft drinks. This study concluded that the role of the mother in influencing children's food patterns should not be underestimated.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

Sales Effectiveness of Selected Advertising and Promotion Techniques for Broilers. Sales data from a 6-week test in two Ohio cities were analyzed to determine effects of week-to-week changes in pricing and merchandising on broiler sales. Fluctuations in broiler movement in sample stores were found to be explainable chiefly by changes in broiler price, display area, and newspaper advertising. These stimuli were found to exert their greatest influence on broiler sales when all were used to feature broilers. Estimates of the effect on sales of price change, amount of display space, and newspaper advertising were made. Additional work is now underway to analyze the principal factors associated with the change in the farm price of broilers over a 10-year period. Broiler advertising lineage in a sample of 60 U. S. newspapers during the second week in each month has been compared with prices

received by Georgia producers during 1954-61. Over 80 percent of the variation in farm prices during this period is explained by broiler supply, cold storage holdings, the wholesale price index, season of the year, and retailer advertising. The analysis of retailer advertising indicates that it tends to be heaviest on a down price. However, it appears that increased retailer advertising does strengthen the farm prices in subsequent months.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Poultry and Eggs

Brown, Sidney E., May 1964. Retail Sales of Broilers and Meat as Affected by Price, Display Area, and Newspaper Advertising, ERS-180, pp. 12.

MARKET STRUCTURE, PRACTICES, MARGINS,
COSTS, AND EFFICIENCY
Marketing Economics Division, ERS

Problem: The purpose of this research is to find solutions for economic problems in marketing dairy, poultry, and meat animals and their products. More specifically, it is to find answers to the needs of farmers, marketing agencies, and the public for economic knowledge about these commodities--needs for economic knowledge that is relevant to marketing decisions and to the shaping of public policy and programs. This project includes studies of margins, costs and efficiency; of the structures of the systems for marketing individual products; and of the methods and practices followed by farmers, marketing firms, and related public agencies. It provides accurate information about the form and working of the marketing system as a basis for initiating desirable changes and for keeping all parts of the system abreast of technological and economic progress.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program of economic research to assist farmers and marketing agencies to adapt to changes in the environment in which they operate. Work in this area is conducted at Washington, D. C. and in cooperation with State agricultural experiment stations at Durham, N. H., Athens, Ga., St. Paul, Minn., Ames, Iowa, Fort Collins, Colo., Stillwater, Okla., and College Station, Texas. The Federal scientific effort devoted to economic research in this area totals 33.3 professional man-years, distributed as follows: dairy 10.0, swine 0.4, beef 0.5, livestock (cross-commodity) 10.7, and poultry and eggs 11.7. By functional areas, it is distributed as follows: structures, practices and competition 15.0, product quality 3.1, information, outlook and rural development 0.7, and margins, costs and efficiency 14.5.

PROGRAM OF STATE EXPERIMENT STATIONS

All the State experiment stations are conducting economics research dealing with the marketing of animals and animal products.

Poultry and egg research in the area of marketing economics includes table eggs, egg products and poultry meat consisting of broilers, heavy young chickens and turkeys. The principal areas of work are market structure, practices and competition, costs, margins and efficiency of assembly, processing, and distribution; merchandising and promotion and transportation, storage and interregional competition. Three regional marketing studies are being conducted. NCM-31 is studying the effects of coordinated production-marketing programs and new marketing technology upon market channels and

institutions. NEM-21 is evaluating the economic feasibility of alternative egg marketing systems relative to market requirements and the competitive position of the industry. SM-26 is studying the market structure of the broiler industry in the South and the impact of national marketing orders on its economic organization and efficiency.

The State effort devoted to poultry and egg marketing research amounts to 32.7 professional man years of which 15.3 is devoted to egg and egg products and 17.4 to poultry.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Structure, Practices, and Competition

1. Pricing Eggs. Egg handlers outside of cities where base price quotations are issued knew less about how quotations are determined than did the egg handlers in those cities. Handlers felt that the base price quotations could be held out of line with true values but only for 2 or 3 days. Transfer of ownership of eggs at various points in the marketing channels is at price differentials below and above the base price quotations, with relatively few at the quotations.

2. Egg Procurement by Large-Volume Distributors. Analysis of large-volume retailers' responses to a survey indicates: (a) Their procurement system for eggs is unique among the commodities handled, (b) long-run decisions are made at the headquarters level while short-run decisions are more apt to be decentralized to a regional or city basis, (c) on the buying side, the basis for arriving at price is changed infrequently, and (d) they leave the problem of balancing supply and demand to their suppliers by ordering only what is needed. Analysis of suppliers' responses indicates: (a) Eggs are the only commodity handled or are very important, (b) changes in business practices to adjust to large volume retailers demands are mainly automatic grading by machines, payment to producers on a grade-yield basis, and lowered margins, (c) they balance supply and demand by supplying the large-volume retailer's needs and selling the balance to other outlets.

B. Product Quality

1. Egg Quality and Marketing Costs. Despite the increased importance of quality-control programs, variation in quality of individual producers' eggs remains substantial. This suggests the need for stricter enforcement of programs or their adjustment to more appropriate standards.

C. Margins, Costs and Efficiency

1. Commercial Egg Industry in the South. Most new technological innovations in egg packing plants are likely to be labor-saving in nature, reducing man-hour requirements. Despite projected increases in factor prices, particularly wage and salary levels, future costs per unit will be reduced. Scale relationships are likely to remain largely unchanged.

More highly integrated firms in the present industry appear to have lower costs in both production and marketing operations. Net returns per unit of product tend to be higher for egg contracting firms and large integrated producers than for cooperative associations and independent receivers. Rate of return on investment was highest for large integrated producers and independent receivers. Differences in all cases are small.

2. Commercial Hatchery Costs. Economies of scale relationships for egg-type chick hatcheries appear similar to those for broiler-chick hatcheries. However, costs per pullet chick produced are more than twice as high for any size hatchery as in producing broiler chicks because of sexing costs, disposal of most cockerel chicks, and the shorter operating season (or lower percent of annual capacity used). For turkey poult hatcheries, most of the potential economies of scale are realized with a hatchery setting about 5 million eggs in a 7 month season.

3. Costs of Assembling and Processing Turkeys. Assembly costs per pound of live turkey tend to rise with firm size due to larger supply areas. Actual costs for firms handling less than 5 million pounds were about 0.3 cents per pound and over 0.5 cents per pound for larger firms. These costs can be reduced slightly by better organization and handling methods, and somewhat more by increasing density of supply areas.

Integration of contract operations with other operations is growing. This is occurring mainly through risk-sharing contracts with feed firms and growing more turkeys on company owned or leased farms. About 20-25 percent of turkey producers secure credit from banks and traditional sources. Production financed by feed companies, processors, and others in the industry amounts to 40-50 percent. Risk-sharing contract production is 25 percent; company production 10-15 percent (above are not completely additive, but overlap to some extent).

4. Transfer Costs for Broilers. Spatial (transfer) costs decline substantially as production density increases from 1,000 to 5,000 pounds per square mile, but the rate of decline between 5,000 and 25,000 pounds per square mile is much less rapid. Total costs for feed milling, chick distribution, live broiler assembly, hatchery and feed mill operation, and processing decline from 6.5 to 5.1 cents per pound of live broiler as integrated organization size increases from a processing plant size equivalent of 600 to 3,600 birds per hour, when density of production is 5,000 pounds per square mile. Larger units experience further but minor reductions in aggregate costs. Costs per ton in milling broiler feeds decline from \$8.61 to \$3.96 per ton as mill size increases from 21 to 348 tons per day when operating at effective annual capacity.

5. Poultry and Egg Margins. Price spreads changed little from 1962 to 1963, but prices have varied at most market levels.

Price specials do have an effect on volume of sales of frying chickens. The price of chickens, however, was the major factor influencing the volume of sales. The price elasticity of demand was -1.75 at the retail level, but less elastic at the farm level.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Poultry

Burbee, C. R., and Bardwell, E. T. May 1964. Marketing New England Poultry. 6. Economies of scale in hatching and costs of distributing broiler chicks. New Hampshire Agri. Expt. Sta. Bul. 483. 56 pp.

Gray, L. R. February 1964. Marketing spreads for eggs, frying chickens, and turkeys in selected cities of the United States. ERS-159. 6 pp.

Gray, L. R. July 1964. Effects of price specials on volume of sales of frying chickens. Agricultural Economics Research, Vol. XVI, No. 3. 5 pp.

Jones, H. B. July 1964. Developing plant facilities for grading and packing eggs. Ga. Expt. Serv. Marketing Report 2-3. 20 pp.

Rogers, G. B., and Rinear, E. H. September 1963. Costs and economies of scale in turkey processing plants. MRR-627. 61 pp.

Rogers, G. B., Henry, W. F., Brown, A. A., and Bardwell, E. T. April 1964. Marketing New England poultry. 5. Effects of firm size and production density on assembly costs. New Hampshire Agri. Expt. Sta. Bul. 482. 62 pp.

June 1964. Bulk delivery of feed in New England. New Hampshire Extension Service. 20 pp.

CONSUMER PREFERENCE AND QUALITY DISCRIMINATION--
HOUSEHOLD AND INDUSTRIAL
Standards and Research Division, SRS

Problem. With the increasing complexity of marketing channels and methods, it has become almost impossible for consumers to express to producers either pleasure or displeasure with available merchandise. To market agricultural products more effectively, it is necessary to understand existing household, institutional, and industrial markets and the reasons behind consumers' decisions to purchase or not to purchase. Information is needed on consumers' attitudes toward old and new product forms of agricultural commodities, preferences, levels of information or misinformation, satisfactions or dislikes, and what product characteristics would better satisfy current consumers and/or attract new ones. It is also important to know the relationship between the consumption of one agricultural commodity and another in consumers' patterns of use, the relationship between agricultural and nonagricultural products, and probable trends in the consumption of farm products. Producer and industry groups as well as marketing agencies consider such information essential in planning programs to maintain and expand markets for agricultural commodities which, in turn, increases returns to growers.

USDA PROGRAM

The Special Surveys Branch conducts applied research among representative samples of industrial, institutional, or household consumers and potential consumers. Such research may be conducted to determine preferences, opinions, buying practices, and use habits with respect to various agricultural commodities; the role of competitive products; acceptance of new or improved products; and consumers' ability to discriminate among selected attributes of a product or levels of an attribute, and the preferences associated with discriminable forms.

In addition to the studies of consumer preference and discrimination, the Branch also provides consultants and conducts special studies, upon request, for other agencies in the USDA or within the Federal Government, when survey methods can be usefully applied to the evaluation of programs, services, or regulatory procedures of interest to the requesting agencies.

The research is carried out in cooperation with other USDA or federal agencies, State experiment stations, departments of agriculture, and land grant colleges, and agricultural producer, processor, and distributor groups. Closely supervised contracts with private research firms are used for nationwide surveys; studies in selected areas are usually conducted by the Washington staff with the assistance of locally recruited personnel.

The Branch maintains all of its research scientists, who are trained in social psychology or other social sciences, in Washington, D. C., which is headquarters for all the research whether it is conducted under contract or directly by the Branch. The Federal scientific effort devoted to research in this area during the past year totaled 7.0 professional man-years. An additional .2 professional man-year was devoted to research conducted under a transfer of funds arrangement.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

Consumer Preference

Poultry. A nationwide survey is being conducted under contract by a private market research firm to ascertain household consumers' preferences, use patterns, and purchasing practices for broilers and turkeys. Questionnaire development and pretesting have been completed; interviewing was completed during the summer of 1964.

The study is similar in some respects to the 1956 USDA study on poultry, and some questions have been repeated from the earlier study to provide information on trends in usage since that date. However, the questionnaire was revised extensively to obtain information on current problems and to reflect the many changes which have occurred in the poultry industry in the past six or seven years. Data from this study will provide insights into the measures which might be taken by the poultry industry to market their products more effectively and to increase demand among household consumers.

COMMODITY SITUATION AND OUTLOOK ANALYSIS
Economic and Statistical Analysis Division, ERS

PROBLEM

Because of the instability of the prices he receives and rapidly changing conditions of agricultural production, the farmer stands in special need of frequent accurate appraisals of his economic prospects if he is to plan and carry out his production and marketing activities in an efficient and profitable way. The typical farmer cannot afford to collect and analyze all the statistical and economic information necessary for making sound production and marketing decisions. It is a goal of the Department to provide the farmer with economic facts and interpretations comparable to those available to business and industry. This is accomplished through a continuous flow of current outlook information, the development of longer range projections of the economic prospects for agricultural commodities, and analyses of the economic implications of existing and proposed programs affecting farm commodities.

USDA AND COOPERATIVE PROGRAM

The program includes the regular publication of 12 commodity outlook reports; holding of the Annual Outlook Conference in Washington in mid-November; participation of commodity specialists at regional and State outlook meetings and at meetings of farm organizations and agricultural industry groups; preparation and publication of special articles bearing on both the short-run and long-run outlook for farm commodities; issuance of comprehensive statistical bulletins containing the principal economic series pertaining to the various commodities; long-range projections of supply of and demand for the major agricultural commodities; and continuing analysis of the impact of existing and proposed alternative farm programs as they affect output, utilization, and prices of these commodities.

Except for a Regional Field Office for Livestock, in Denver, Colorado, all the USDA situation and outlook work is carried on in Washington. The regional livestock project is a cooperative effort including the Economic and Statistical Analysis Division, the Federal Extension Service, and State Extension Services in the Western and certain Great Plains States.

The total USDA commodity situation and outlook program currently involves 21.5 professional man-years.

Poultry. This work involves 1.5 professional man-years in Washington. The outlook and situation program provides a continuing appraisal of the current and prospective economic situation of poultry and eggs. Appraisals

are published 5 times a year in the Poultry and Egg Situation, and quarterly in the Demand and Price Situation and the National Food Situation. A comprehensive analysis of the poultry and egg situation is presented at the Annual Outlook Conference. Appraisals also are presented at regional or State outlook meetings, at meetings of farm organizations and to various agricultural industry groups. Special analyses are prepared on the probable effect of proposed programs on the price, supply and consumption of poultry and poultry products. Basic statistical series are developed, maintained, improved and published for general use in statistical and economic analysis.

PROGRAM OF STATE EXPERIMENT STATIONS

For the most part the States depend upon the U.S. Department of Agriculture for the yearly across-the-board commodity situation and outlook research. The State extension staff members supplement and adapt such research information to meet the commodity situation of their States.

Four States have projects that deal specifically with analysis of current price trends and prediction of future prices. There is increasing interest in longer range price prediction because of the growing specialization of farms, which make yearly enterprise shifts less common and less feasible, and which calls for large capital commitments over longer periods of time.

The total direct research effort in the situation and outlook area is approximately 1.7 professional man-years. While not designated as outlook research, much of the research conducted by the experiment stations and reported elsewhere contributes to improved understanding of price-making forces, which in turn improves market situation analysis and price forecasting.

PROGRESS--USDA AND COOPERATIVE PROGRAMS

Poultry and Eggs

In cooperation with specialists in other parts of the Department, considerable effort was directed to analyzing the impact of Government purchase programs for eggs and turkeys, and to projecting probable future trends in the egg industry. A cyclical approach to analyzing the supply response in the broiler industry, initiated a year earlier, received further attention in 1963-64. It proved to be of great value in providing a better understanding of the forces that generated over-production and low prices in the broiler industry in late 1963 and early 1964. The results of this analysis, which were kept current, were reported several times in the Poultry and Egg Situation; in January 1964 the results were presented to a top-level broiler industry group organized under the auspices of the Institute of American Poultry Industries. The analysis also received extensive coverage in the poultry press. Such information is useful to the industry in efforts to moderate cycles of over-production and low prices.

PUBLICATIONS--USDA AND COOPERATIVE PROGRAMS

Poultry and Eggs

Bluestone, H. Poultry and Egg Situation. Published 6 times a year. ERS, USDA, Washington, D. C.

Bluestone, H. November 1963. Projections to 1968 for the poultry industry. Poultry and Egg Situation, pp. 19-21.

Bluestone, H. February 1964. The role of industry statistics. Poultry Processing and Marketing, a Watt Publication, pp. 22-23, 28-63.

Stoddard, E. O. January 1964. Costs and returns on Georgia broiler farms, 1962. Poultry and Egg Situation, pp. 14-21.

